Host-Parasite Relationships of the CLOVER CYST NEMATODE, *Heterodera trifolii* Goffart

By REINHOLD MANKAU and M. B. LINFORD

BULLETIN 667

UNIVERSITY OF ILLINOIS · AGRICULTURAL EXPERIMENT STATION

CONTENTS

Urbono, Illinois December, 1960

Publicotions in the bulletin series report the results of investigotions mode or sponsored by the Experiment Stotion

Host-Parasite Relationships of the Clover Cyst Nematode, *Heterodera trifolii* Goffart

REINHOLD MANKAU[1] and M. B. LINFORD[2]

DESPITE THE VAST ECONOMIC IMPORTANCE OF many plant-parasitic nematodes and the efforts currently being made to understand their ecology and to devise or improve methods for controlling them, relatively little attention has been given to how these pathogenic worms obtain their food and how they incite disease in plants. In 1887 the occurrence of groups of large, multinucleate *giant cells* was reported in association with the nematode head in galls caused by root-knot nematodes, Meloidogyne spp. (33).[3] These obviously were a result of the feeding process. Later, masses of pathologically altered host tissue were reported to develop adjacent to heads of cyst-forming species of Heterodera. These often have been called giant cells, which they resemble superficially. In this paper, however, they are designated *syncytia,* a term used by Němec (27) that is more aptly descriptive and that avoids confusion with the giant cells associated with Meloidogyne.

Several studies of the development of giant cells and of syncytia were made before the basic mechanics of feeding by the nematodes were understood. This resulted in some erroneous interpretations of how the nematodes incite pathological changes and how they obtain their food. One or more species of Meloidogyne, which today cannot be identified because of subsequent taxonomic revision, were studied more fully in these respects than were the members of the genus Heterodera. Both genera have been studied chiefly in association with highly suitable host plants.

This bulletin reports investigations of the clover cyst nematode, *Heterodera trifolii* Goffart (11), in association with suitable hosts and with less suitable, resistant, and immune plants. These studies were undertaken to determine how the nematode enters the root, how it

[1] REINHOLD MANKAU, Assistant Nematologist, Department of Plant Nematology, University of California, Riverside, California, formerly Research Assistant, Department of Plant Pathology, University of Illinois. The material in this publication is a portion of the dissertation submitted by the senior author to the Graduate College, University of Illinois, in partial fulfillment of the requirements for the Ph.D. degree.

[2] M. B. LINFORD, formerly Professor of Plant Pathology at the University of Illinois. Dr. Linford, who was internationally recognized as an authority on nematodes, died in September, 1960, shortly before this bulletin went to press.

[3] Figures in parentheses refer to literature citations on pages 49 and 50.

obtains its food, how it injures the plant, and what factors determine its ability to thrive in some plants and not in others. Some of the major conclusions have been published in an abstract (21).

This nematode appears less important economically than several other species of the genus, and when it attacks clovers in mixed stands with grasses, its pathogenic potential is easy to underestimate. In the greenhouse, however, when a few cysts are added to sterilized soil in which such hosts are growing, it multiplies rapidly and within a few months severely weakens or kills the plants. Evidently, therefore, it is a representative species of its genus with respect to pathogenicity. It is also similar to some related species in its morphology and life history and in the grosser aspect of its relationship to host plants. The ease of maintaining it in greenhouse culture and the inclusion within its host range of plants which also are hosts of other species of the genus, made it a suitable parasite for this study.

LITERATURE REVIEW

The entry of larvae of *Heterodera schachtii* Schmidt into roots of sugar beet on agar was investigated by Berliner and Busch (1) who concluded that penetration of the epidermis could occur only through wounds. Sengbusch (32), however, after studying living as well as fixed and sectioned material, reported that the larva uses its stylet to weaken the walls of cortical cells until it can force its head through, and suggested that, with the support offered by soil, larvae should be able to break into the unwounded epidermis. Linford (19) found that larvae of Meloidogyne sp., after a period of feeding on epidermal or dermatogen cells, used the stylet to weaken the outer wall of one of those cells until the head could be forced into it, and then again to weaken the inner wall of the cell. Where fresh wounds were available, however, they were entered freely by these larvae.

The way species of Heterodera and Meloidogyne feed and incite pathological disturbances near the head long remained obscure, partly at least because of the limitations of histological methods and the technical difficulties inherent in microscopic observations of living parasites within roots. Němec (26), Kostoff and Kendall (14), and Christie (4), failing to find evidence that the stylet of Meloidogyne ever punctured cells, assumed that the nematode poured secretions into intercellular spaces around its head and that these secretions altered the permeability of these cells in such a way that nutrients escaped from them into intercellular spaces where the nematode was able to suck them up. Němec (27) extended his hypothesis of a

nectary-like function, to the syncytia associated with *H. schachtii.* Sengbusch (32), however, observing live *H. schachtii* within roots and live females dissected from roots and mounted in water, saw the stylet thrust so persistently that he was convinced it must be used in the process of feeding. In paraffin sections he sometimes found the stylet tip protruding through a boundary wall into the syncytium. Němec (28) failed to confirm Sengbusch's findings and restated his earlier interpretation.

Reconsideration of the feeding process in Meloidogyne followed observations by Linford and Oliveira (20) and Linford (15) on several mycophagous and predaceous nematodes that were especially suitable for study in the living condition. These nematodes were seen to thrust the stylet forcibly into the hyphal cell or into the body of the prey and hold it inserted during the entire period of ingestion. This ingestion was seen to occur only during periods of pulsation[1] of the median bulb of the esophagus. Also, among predaceous species of Aphelenchoides Fischer, saliva[2] was seen to be injected through the stylet into the prey where it had two observed functions: paralysis of the prey and extra-oral digestion.

In detailed studies of living Meloidogyne sp., utilizing several methods of observation, Linford (16, 19) found that larvae and females use the stylet in feeding. Larvae were seen to insert the stylet tip into a cell both at the root surface and during intercellular wandering, and then to lie apparently motionless for a few seconds before beginning a short period of pulsation of the median bulb. Cessation of this pulsation was followed by prompt retraction of the stylet. Enlarged females, despite their loss of mobility, were seen to retain ability to turn the head[3] through wide angles and to thrust the stylet into cells surrounding the head. Females dissected alive from the root were seen to extrude saliva from the protruded stylet and, when they were

[1] Pulsation of the median bulb is the expression used throughout this paper to report a rhythmic muscular activity that opens and closes the valvular mechanism within the bulb. Muscle fibers connecting from the refractive valve plates to the periphery of the bulb, as they contract and relax, may impart a throbbing motion to the entire bulb and sometimes causes visible motion in nearby parts of the nematode. It is the opening and closing of the valve that is most readily seen. This also is what actuates the intake of food, yet details of the valve action seem not to have been analyzed.

[2] *Saliva,* as the term is used in this paper, designates secretions from the dorsal esophageal gland that pour into the lumen of the esophagus at some point anterior to the valvular mechanism of the median bulb.

[3] Although the *head* of a nematode is not a distinct morphological entity, the term sometimes has been used in the past and will be used in this paper to designate the anterior part of the nematode including the labial region and a variable length behind it, amounting at least to a length equal to that of the stylet.

mounted in a dextrose-peptone solution, they exhibited pulsation of the median bulb only while the stylet was protruded. From these and other observations Linford concluded that the feeding process in Meloidogyne involves both injection of saliva into living host cells and the sucking out of food through the protruded stylet, and that these processes provide the stimuli that cause development of the giant cells upon which the enlarging nematode depends for a progressively increasing supply of food.

The characteristics and development of the giant cells that arise adjacent to the head of Meloidogyne have been described by several investigators since Treub (33) first called attention to their occurrence, and special attention has been given to the question of how these cells attain the multinucleate condition. Little new has been published since Christie (4) reviewed this literature in connection with his own thorough study of the development of root-knot nematode galls: therefore no detailed review is needed here. Usually from 3 to 6 very large, elongated, densely protoplasmic, multinucleate cells develop in a compact group, with one end surrounding the nematode head. These develop progressively for many days by the dissolution of cell walls and the coalescing of protoplasts. Despite the progressive incorporation of neighboring cells into these giant cells, the latter tend to retain their individuality. Some workers have reported dissolution of walls between neighboring giant cells, but Christie (4) reported that he had not seen an instance in which all the giant cells had united to form a single unit.

It is the consensus of recent investigators that the multinucleate condition results chiefly from the pooling of nuclei from the coalescing cells, although several workers have reported some mitotic divisions in young giant cells. Nuclei in these pathologically altered cells early become abnormal in size and appearance, but what was once reported to be amitosis and nuclear fragmentation in older cells is now generally interpreted to be coalescence of degenerating nuclei.

Němec (28) in his second paper on the development of syncytia adjacent to the head of *Heterodera schachtii* in beet roots, reported the dissolution of the cell walls first caused holes and finally caused the entire walls to dissolve, allowing adjacent protoplasts to merge until finally the syncytium was one continuous mass. Although he reiterated the view that the syncytium acted as a nectary in secreting nutrient substances to be sucked up by the nematode, he recognized that the parasite, in the beginning, must modify the host tissues by chemical means. Having found nuclei that appeared to have forced small protuberances through holes in cell walls, he suggested that nuclei may be

active in causing wall dissolution. He found neither coalescence nor division of nuclei in syncytia, although he had earlier (26) observed mitoses in developing giant cells associated with Meloidogyne sp.

METHODS AND MATERIALS

The nematodes. The clover cyst nematodes used in this study were from greenhouse subcultures of a population collected from Champaign county, Illinois, and studied by Gerdemann and Linford (10). These cultures had been maintained continuously in pots or flats with *Trifolium repens* L., varieties Dutch White and Ladino. *Heterodera trifolii* is the preferred name of this nematode although it has been mentioned frequently in the literature also as *H. schachtii* var. *trifolii.* No males were seen in this population at any time.

The larvae used in this investigation were hatched from cysts removed from greenhouse subcultures of Ladino clover. Cysts were placed on a fine cloth sieve held on the surface of charcoal-treated distilled water in a shallow dish. The larvae passed through the cloth and were collected from the water below.

Two other species of nematodes were studied to a limited degree. *Heterodera schachtii* Schmidt was obtained from greenhouse subcultures of material from an infested sugar-beet field in southeastern Wisconsin. *Meloidogyne hapla* Chitwood, 1949, was obtained from greenhouse subcultures of nematodes from clover from the same source as the *H. trifolii.*

Experimental infestations. To obtain material of known age for cytological and histological study, Ladino clover was seeded in sand in 4-inch pots and allowed to grow 20 days. Nutrient solution was applied occasionally to insure good growth. On the 21st day the pots were infested with a water suspension of several hundred larvae, distributed as evenly as possible over the surface of the sand and watered in. After 24 hours, each pot was placed in a pan of water and the seedlings were floated free with a gentle stream of water. They were rinsed well to remove larvae from the root surface, and repotted in 4-inch pots of a steamed mixture of soil, sand, and rotted manure, with 3 or 4 plants per pot. At intervals of 1 to 4 days, seedlings from each of two pots were carefully washed free of soil and either fixed and processed for paraffin sectioning or stained and cleared as whole mounts.

Developmental stages in pea, *Pisum sativum* L., and in red clover, *Trifolium pratense* L., were obtained in the same manner. The pea was a canners' strain of Perfection while the red clover was the composite variety Midland. In another series, peas were seeded in soil in

4-inch pots and grown until several leaves appeared, then infested by adding about 200 larvae to each pot. Plants were then removed at 48-hour intervals for fixation or for processing as whole mounts.

Several other species and varieties of plants known or suspected to be less suitable, or resistant, to this nematode were tested by planting the seed directly in a heavily infested mixture of sand and soil. All Ladino clover planted in aliquots of this mixture, before and during tests of other plants, became infested. The following plants were tested by growing them separately in several pots of this mixture: cabbage (*Brassica oleracea* L.), carrot (*Daucus carota* L.), sugar beet (*Beta vulgaris* L.), wheat (*Triticum vulgare* Vill.), lespedeza (*Lespedeza cuneata* G. Don.), and yellow sweet clover (*Melilotus officinalis* (L.) Lam.). After a period of time sufficient for several generations of the nematode to develop in Ladino clover, the roots were washed and examined for cysts or females. If any nematodes were observed on the roots, the portions containing them were excised and fixed. Some plants other than those just listed (see page 40) were tested only by growing them in flats of infested soil from which Ladino clover had recently been removed. A well-mixed batch of soil was used for more extensive tests of 27 varieties of soybeans, *Glycine max.* (L.) Merrill, representing all maturity groups from 0 through VIII. Seed was provided by Dr. D. W. Chamberlain, U. S. Regional Soybean Laboratory, Urbana, Illinois.

The 19 soybean varieties of groups 0 to IV were planted May 2, 1955, with three seeds per 4-inch pot and two pots per variety. One plant was removed from each pot after 18 days, and its roots processed with acid fuchsin in lactophenol to determine whether larvae had entered. The remaining plants were removed 36 days later, and their roots washed and examined for both cysts and larvae. Loose cysts were collected on a No. 60 sieve and larvae on a No. 325 sieve. The entire root mass, spread in water, was examined under a stereoscopic microscope, then turned over and examined again. Selected parts of each root system were cut out, spread more thinly, and examined in detail; and other selected parts were stained and cleared to permit detection of parasites within the tissues. Histological preparations were made from rootlets of Dunfield and Earlyana varieties bearing enlarged females or cysts.

The 8 varieties of groups V to VIII were planted July 11, 1955, and examined after 44 days. Methods of examination were the same except that staining and clearing with acid fuchsin in lactophenol was omitted with five varieties.

All cultures except those specified in the following lines were grown in a greenhouse where temperatures varied between 21° and 27° C. To test the development of *H. trifolii* at lower temperatures, 75 cysts per 6-inch pot were added to young Ladino clover in steamed soil maintained at approximately 15° to 17° C. under fluorescent lights. Because of limited space, only three pots were infested and one served as a check. After 6 months the roots were washed and examined for nematodes, and sections were processed for cytological study.

Processing for microscopic study. For whole mounts, roots were processed in boiling lactophenol with acid fuchsin, rinsed in distilled water, then stored and examined in clear lactophenol. Roots for the study of developmental stages in Ladino clover, red clover, and pea were fixed in Karpechenko's solution following Rawlins (30) and dehydrated in an ethyl-tertiary butyl alcohol series. In the 70 percent TBA solution, the roots were sufficiently cleared that the nematodes, darkened by the fixation, could be seen under a stereoscopic microscope. Parts of roots containing parasites and judged suitable for study were selected and cut to lengths favorable for further processing and embedding in paraffin. Most of the remaining material was fixed in a formalin-acetic acid-alcohol solution (13, page 41). Some pea roots were fixed in Randolph's modification of Navashin's fluid (13, page 45).

The stains employed with paraffin sections were the safranin-fast green combination, Heidenhain's iron haematoxylin and safranin combination, Fleming's triple stain following Johansen (13), and chlorazol black E following Darrow (6). Some slides were stained by the Feulgen reaction as described by Darlington and LaCour (5). The safranin-fast green combination and especially the chlorazol black E stain were excellent for demonstrating alterations in cell walls as well as protoplasmic details.

Observation of living material. The penetration of roots by living larvae was observed with minature root-observation boxes (17, 18) in which young Ladino clover seedlings were grown against the cover glass on one side of the box. After the seedlings had produced root growth adequate for observation, larvae were introduced in a water suspension. Activities of the larvae were then followed by placing the box on the stage of the compound microscope and viewing through the cover glass with the low power objective or with a 40× water-immersion lens following procedures described by Linford (18).

Living females were dissected from roots in water with care to avoid injuring them and were observed in water without a coverslip, using a 40× water-immersion objective.

ACTIVITIES OF THE LIVING NEMATODE

Entry into roots. The second-stage larvae of *H. trifolii,* as seen in root-observation boxes containing young Ladino clover (Fig. 1), moved freely through moist soil and along roots, definitely propelling themselves and not being dependent upon movements of water films or growth of roots toward them as sometimes has been suggested. When the larvae moved along a root, they often thrust the stylet slowly and irregularly against the epidermis.

Entry of a larva into a root, except where a pre-existing wound facilitated the process, was accomplished only after a larva had thrust its stylet forcibly and persistently against an outer epidermal wall in a way that evidently weakened that wall. For this the body of the larva had to be well supported by surrounding soil or, in observation boxes, by the glass coverslip, because the stylet was applied with such force that the head was seen to recoil. During this process the nematode head was sometimes seen to slip away from the small area being battered. The larvae returned to the same spot or moved to a new location. This thrusting commonly was at a rate approximating 80 thrusts per 30 seconds and sometimes continued for as long as two minutes. Sometimes the stylet tip was seen to pass through the wall of the epidermal cell, but no larva was observed to feed from the root surface.

Larvae of *Heterodera trifolii* associated with a rootlet of Ladino clover as seen in an observation box. Some larvae were penetrating. Photographed by incident light. ×155. (Fig. 1)

After the outer wall was sufficiently weakened, pressure exerted by the larva forced the head into that cell, causing the cell's disruption and death. The head was then forced against the inner wall and much the same process was repeated. This wall commonly was broken through more rapidly, allowing the head to be forced into the subepidermal cell. After this, entry of the larva full length into the cortex was relatively rapid: one was seen to move half its length into the cortex within 5 minutes and to be entirely inside 11 minutes later. Others were also seen to penetrate in about 15 minutes. The process of entry was in short stages, comparable to cell lengths, suggesting that the larva was delayed slightly by each wall that it broke through, for the entry was entirely intracellular. In later stages of entry, the larvae sometimes began to feed and this retarded their locomotion. In the outer cortex, however, there seemed to be no feeding: the esophageal bulb has been observed carefully during passage of the head through the outer layers of the cortex without once being seen to pulsate.

The fresh wound made by the entry of a larva was definitely attractive to others that utilized it for more easy entry. After one larva was seen to enter, three others in turn entered through the same epidermal cell but then apparently broke new paths through the cortex. These each required about 15 minutes to move full length into the root. Three more larvae then were seen to enter more rapidly, using not only the hole in the epidermal cell but also the tunnels in the cortex made by preceding larvae. By this time, still more larvae had collected around the surface wound. On another root, approximately 50 larvae were observed congregated around a wound while a few were distributed over unwounded surfaces in the immediate vicinity. Larvae wandering along a root surface were seen to increase the frequency and forcefulness of stylet thrusting as they approached a wound.

The wound made in epidermis and cortex by a single larva was never very large nor conspicuous. Where high concentrations of larvae occurred, however, the persistent entry of additional larvae through an enlarging wound sometimes resulted in the formation of cavities that filled with fluid and in which infusoria and even rotifers were seen.

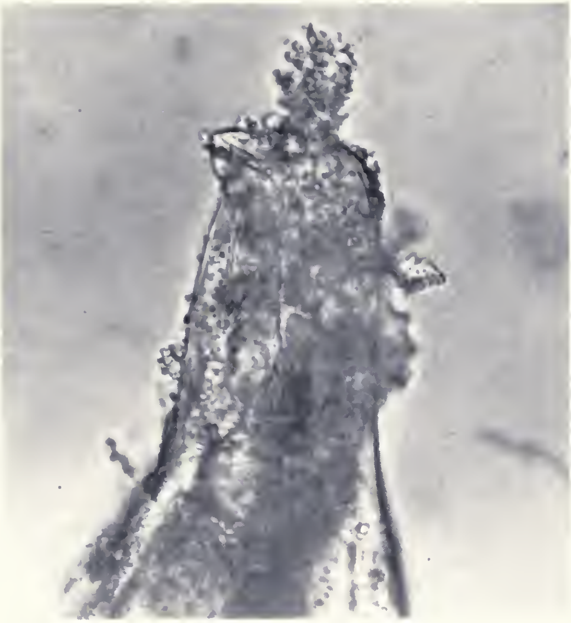
Feeding by larvae within the cortex. Although *H. trifolii* larvae were not seen feeding on the epidermis or during penetration through the first few cortical cells, some larvae did begin to feed while still wandering within the cortex. Occasionally, feeding occurred near enough to the root surface that both stylet and median bulb could be seen. Under these conditions, however, it was sometimes difficult to determine whether stylet thrusting was preliminary to feeding or to the

rupture of a wall for further penetration. The stylet was sometimes thrust with sufficient force that the wall was seen to give; sometimes the stylet bent visibly; and sometimes, if the nematode head was not braced adequately and the stylet was not thrust almost perpendicularly to the wall, the blows glanced off. Feeding was observed only while the stylet tip was inserted through a wall into a living cell. Pulsation of the median bulb appeared to start as soon as the stylet tip was inserted and to stop only when it was ready to be withdrawn. Repeated feeding from the same cell was not observed, but sometimes a larva was seen to feed from more than one adjoining cell before advancing to force its head into another cell. Cells were not always fed from before the larva weakened a wall by battering with its stylet and then forced its head through. Larvae that were seen moving rapidly through the cortex and evidently following passages opened by earlier invaders, were not seen to feed.

Feeding by maturing larvae and by females. Adult females and third- and fourth-stage larvae, dissected from the roots of a number of hosts, exhibited a rhythmic thrusting of the stylet and pulsation of the esophageal bulb. These activities also were observed within slices of living roots under water, but optical difficulties presented by root tissues made an accurate determination of the sequence and details of feeding activity impossible. Fig. 2 is an example of an immature female observed in water after dissection from a pea root. This nematode was observed for 48 hours before being discarded, and it maintained rhythmic alternations of stylet thrusts and bulb pulsations during this entire period. Other females were observed for periods ranging from a few minutes to over 48 hours.

When a third- or fourth-stage larva or a young female was dissected from a root, the activity of the stylet might temporarily be stopped because of manipulation, but it usually was resumed by the time the specimen could be arranged for examination at high magnification, or the stylet soon became active with an irregular pattern and rate of thrusting. Some of the thrusts barely brought the stylet tip through the oral opening, while others protruded it to the limit imposed by the labial framework. Thrusts sometimes were paired, two quick thrusts followed by a longer interval before the next thrust, as described by Sengbusch (32) for *H. schachtii,* but actual counts of paired and unpaired thrusts failed to show either to be preponderant.

The rate of thrusting exhibited was irregular. It varied within 60 seconds by alternate periods of vigorous and rapid thrusts and periods in which thrusting was weak and infrequent. In immature females the



Anterior part of a fourth-stage larval female of *Heterodera trifolii* that was observed in water for 48 hours after dissection from a pea root. The knobbed stylet base, lumen of the esophagus, median bulb, and valve at the center of that bulb are visible. In the living specimen, the entire stylet and the salivary reservoir at the left of the lumen of the esophagus could be seen with ease. Debris partly obscures body contours. ×480. (Fig. 2)

thrusts were more vigorous and rapid, and the rate for consecutive 60-second periods varied more than for the regular pattern of mature females. One female, approaching maturity, displayed an average rate of 25 to 26 thrusts per minute.

A very limited, barely discernible movement of the head of immature females was observed, involving only about the first 6 to 8 annules. The ability to move the head was gradually lost as development proceeded through larval stages to fully mature females which did not move the head. The heads of mature Meloidogyne females, on the contrary, remained very mobile within the root (16).

At periodic intervals, females in water projected the stylet tip to its limit and began a pulsation of the esophageal bulb. The stylet remained protruded during the entire period of pulsation and was retracted after pulsation had ceased. Periods of pulsation were equally long in individual females and were separated by equally long periods of stylet

thrusting. From one individual to another, however, the characteristic duration of pulsation varied from a few seconds to several minutes and was generally longer in mature than immature females. One female, which was removed from clover grown at 15° to 17° C., had one period of bulb pulsation which lasted 25 minutes. Occasionally these long periods were interrupted by several jabs of the stylet and then resumed. Stylet thrusts became increasingly frequent just prior to the onset of bulb pulsation and were resumed again gradually after it ceased.

The periodic pattern of bulb pulsation alternating with periods of stylet thrusting was maintained by females in water for long periods with remarkably little deviation in duration of periods. Females placed under refrigeration (5° to 7° C.) overnight became inactive but returned to the pattern recorded for the previous day after they warmed to room temperature.

Pulsation of the esophageal bulb was stronger and more rapid in females approaching maturity than in younger stages. Both immature and mature females dissected from roots of clover grown at 15° to 17° C. exhibited a conspicuously stronger and more rapid pulsation than those from plants grown at 21° to 27° C.

THE DEVELOPMENT OF *H. TRIFOLII* IN LADINO CLOVER AND THE CYTOLOGY OF SYNCYTIA

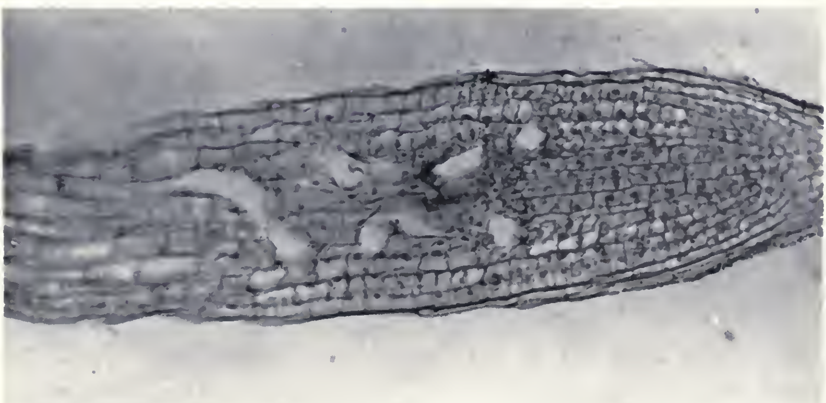
Life history. Slides prepared for cytological studies also proved useful for determining the rate of development of the nematodes because the slides were made from roots that had been fixed a known number of days following entry by the second-stage larvae. The second molt was observed 4 days after the larvae had penetrated. By the sixth day almost all larvae were in the third stage, and by the eighth day some were undergoing the third molt. After 14 days most larvae were in the fourth stage, and some were undergoing the fourth molt. After 17 days many females were fully developed. Some contained the first eggs after 22 days, some were well filled with eggs after 28 days, and a few were turning brown after 33 days. Development was distinctly faster than found by Mulvey (24) at lower temperatures.

Occurrence of the first molt within the egg was confirmed by the observation of molting larvae within eggs still enclosed within the female. Gerdemann and Linford (10) observed it for this species in eggs deposited by the female. Raski (29) observed similar molting within the egg of *H. schachtii.* This was also observed by Hagemeyer (12) for *H. rostochiensis.* Nagakura (25) reported a similar molt within the egg of a root-knot nematode.

Larvae in processed roots. Studies of sectioned roots and of whole mounts indicated that larvae generally penetrated in the younger root-hair zone but that they could enter the roots almost anywhere. Entry directly into the apical meristematic region behind the root cap was observed occasionally. Larvae often were located at the base of a branch rootlet where entry probably was gained through the separation of cortical cells caused by the emergence of the lateral rootlet.

Once a larva entered a root it might establish its permanent site almost at once, or it might wander extensively, leaving a tunnel of broken cells that was clearly visible in the younger tissues (Fig. 3). In slender Ladino clover roots, larvae sometimes penetrated almost directly to the stele and assumed a permanent feeding position with the posterior portion of their bodies still projecting from the root surface. Most larvae penetrated full length, moving away from the root tip. Some, however, moved toward the tip. These sometimes entered the apical meristem where they evidently found unfavorable conditions, turned around, and moved back into older tissue. Establishment of the parasite in a completely undifferentiated zone was not observed. Large numbers of larvae sometimes entered a root close together, as through a wound, but they then evidently dispersed or killed the root because no compact groups of developing females were observed.

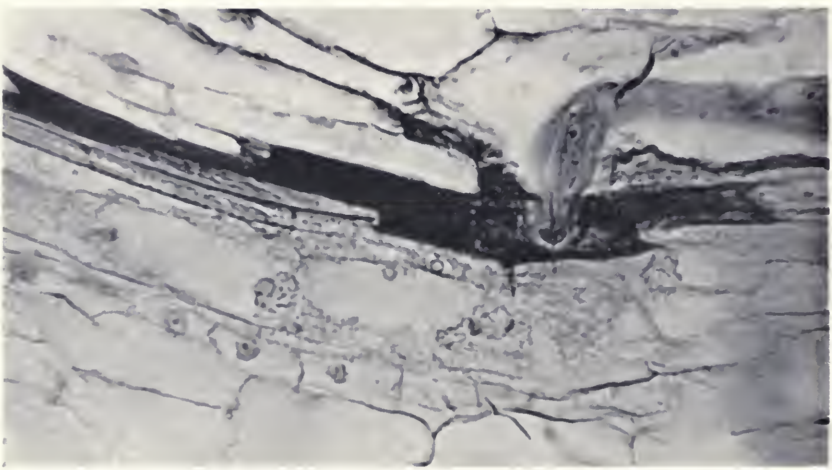
Pathological consequences of wounds made by larvae during penetration and wandering seemed somewhat inversely proportionate to the maturity of the tissues involved. In the cortex of the young root-hair zone and in older tissue, the larvae left a path of broken cells,



Tunnels made by wandering larvae in the elongating zone of a clover root. This much internal wounding usually caused the root tip to die. ×240. (Fig. 3)

sometimes surrounded by a few additional necrotic cells, but under the conditions of these studies, such wounds did not appear to interfere seriously with root function. In the elongating zone, however, larvae passed through the immature central cylinder as well as through the cortex, and this appeared to retard the activity of the apical meristem. It was the occasional larva that passed through the apical meristem itself, however, that appeared to do the greatest mechanical damage, for it broke a path that killed more cells than it actually passed through and seemed to disrupt the organization of the meristem. When several nematodes moved about through immature tissues of a root, the tip might be killed and yet contain no larvae after necrosis was detected, the larvae having already moved up into the maturing zone of the root more suitable for their establishment. Larvae that had established at permanent sites were unable to move on, and they died if tissues around the head became necrotic. Vigorous lateral roots sometimes developed behind necrotic tips.

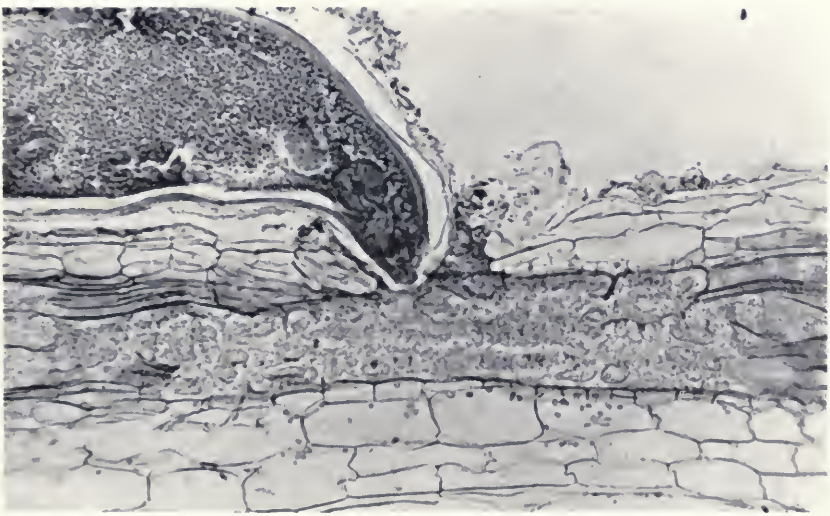
When a nematode finally established at a permanent site in roots in which no secondary tissue had formed, its head, almost without exception, was in a broken endodermal cell or cortical cell near the endodermis (Fig. 4). When feeding, it thrust its stylet through the inner wall of that cell. Some cells of the endodermis and cortex adjoining the head often became necrotic, but the stylet tip always was inserted



Young syncytium in Ladino clover associated with an *Heterodera trifolii* larva four days after entry. The nematode head lay within a broken and deeply stained endodermal cell, and its stylet extended through the cell wall into the syncytium. Many cell walls within the stele had dissolved near the stylet, and mild effects were visible 500 microns away. ×540. (Fig. 4)

into a living cell. The body generally was entirely within the cortex in varied positions but usually oriented nearly parallel to the root axis and close to the stele, with its anterior end pointing away from the root tip and with the neck region bent sharply toward the stele. Occasionally larvae lay partly within the stele.

The syncytium. The syncytium caused by the feeding of *H. trifolii* in the young parts of roots, where no secondary growth had occurred, became a dense, deeply staining, multinucleate, protoplasmic mass consisting of what earlier had been numerous cells. It was formed by a progressive dissolution of cell walls and a merging of protoplasts. These changes began in a single cell fed from by the nematode and rapidly extended into surrounding tissues. The syncytium typically was situated entirely within the stele and was limited on the side toward the nematode by the endodermis or, occasionally, in part by cortical cells. At its ends, the enlarging syncytium was poorly delimited, merging imperceptibly with normal tissue. In the parts of a rootlet that were very young when the syncytium was initiated, it usually occupied the entire cross section of the stele in the latitude of the head of the nematode (Fig. 5), and here it contained almost no intact cell walls



Well developed syncytium associated with a female *Heterodera trifolii* shown in a longitudinal section of a Ladino clover rootlet processed 21 days after the entry of the nematode. The syncytium included the entire width of the stele and was delimited by the endodermis. Cell division in the cortex or in the pericycle formed a periderm-like tissue between the nematode's body and the stele. ×240. (Fig. 5)

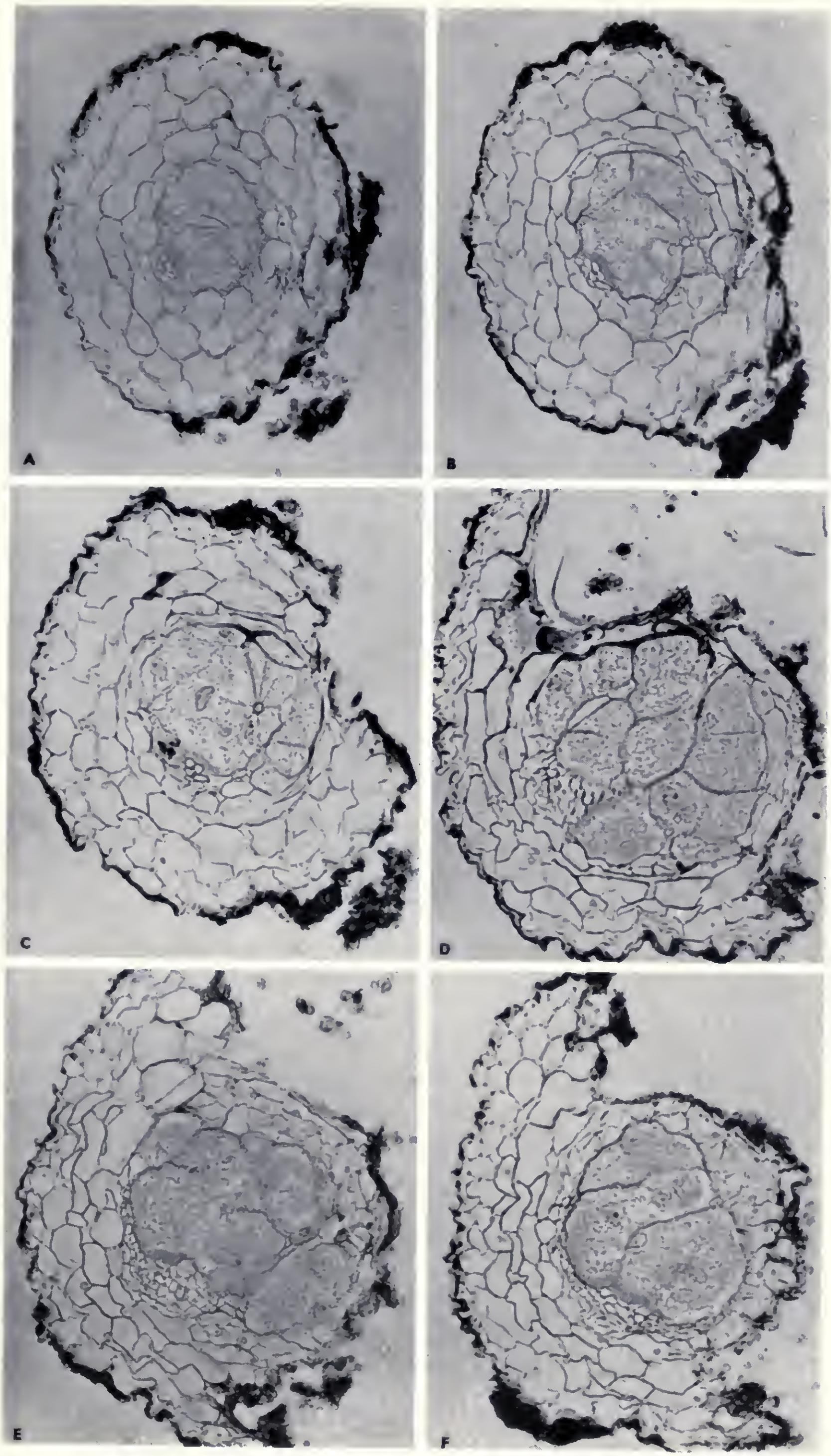
although discontinuous fragments of walls sometimes persisted. Farther from the nematode head, the syncytium was narrower and cell wall dissolution was less complete. In roots of greater diameter and in older roots, the syncytium commonly occupied only a part of the cross section of the stele. The shape of a syncytium in longitudinal section was variable, but frequently it was somewhat lanceolate, broad at one end and narrowing to a fine point at the end farthest from the point of stylet insertion. A length of 2 mm. sometimes was attained, but most syncytia were somewhat shorter. The cell wall through which the nematode stylet protruded usually became thick.

The term *syncytium* is more accurately descriptive than *giant cell* or *giant cell complex,* terms which imply that the pathology results from the growth and enlargement of one or more cells. In individual transverse sections, the protoplasmic mass of the syncytium often appeared to be partitioned by occasional cross walls, giving the impression that it was composed of a number of cells. Study of developmental stages in serial transverse and longitudinal sections, however, indicated that these walls were never continuous but were undissolved portions of the more resistant walls within the stele (Figs. 6, 7, 8).

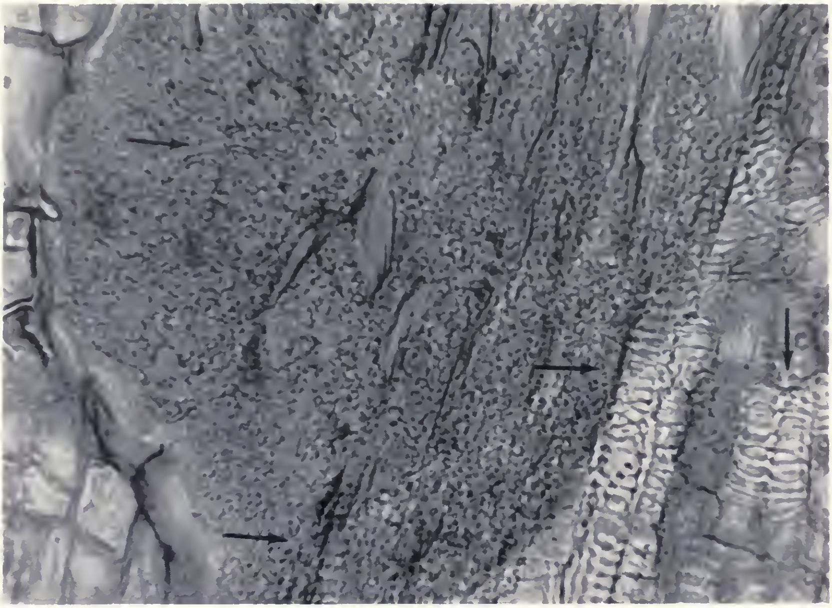
Early stages in development of syncytium. Once a larva began feeding at its permanent site, it quickly caused characteristic changes in the cell fed from, conveniently called the initial cell of the syncytium. The nucleus hypertrophied at once, while the cytoplasm more slowly became granular and hyperchromatic and accumulated near the point of feeding. Very soon the nuclei of adjoining cells also became hypertrophied and their walls began to dissolve, allowing their protoplasts to merge with each other and with the initial cell. The wall through which the nematode fed, however, remained intact and usually gradually thickened. Similar hypertrophy of nuclei, changes in cytoplasm, and dissolution of walls extended progressively outward from the point of initiation, enlarging the syncytium more rapidly in the longitudinal than the radial direction. During these changes, very little if any hypertrophy of host cells occurred around the nematode's body. Cells most quickly merging in the syncytium included chiefly protophloem

---

Variable appearance of a syncytium when viewed in different transverse sections of the stele of a Ladino clover root processed 22 days after the entry of the nematode. A was about 250 microns from the distal end of the syncytium; A, B, and C were 50 microns apart; C was 50 microns from the head of the nematode and 200 microns from D; D, E, and F were 50 microns apart; and F was little more than 50 microns from the proximal end of the syncytium. ×320. (Fig. 6)



(Fig. 6. See opposite page for legend.)

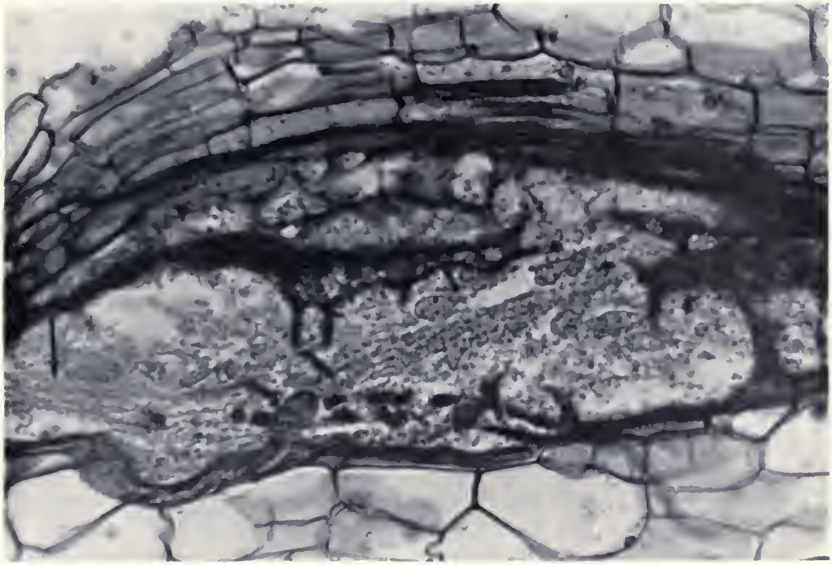


Part of a densely granular syncytium in a longitudinal section of a Ladino clover rootlet that evidently had begun secondary thickening at the time it was invaded by the nematode, 17 days before processing. Cell wall dissolution is most advanced at the left, which an adjacent section showed was near the head of the nematode. At bottom center, the syncytium has expanded into a xylem bundle, and, at the right (two arrows), scalariform thickening of pericycle cells may be seen. Tubular filaments appear at upper left. ×720. (Fig. 7)

and phloem parenchyma but also some immature xylem. Figs. 18 and 19 illustrate early stages in development of syncytia in the cortex of red clover where the parasite was sometimes able to initiate syncytia.

Dissolution of cell walls was at first uneven and incomplete, allowing the cytoplasm of adjacent cells to mingle with that of the initial syncytial cell while the enlarging nuclei might be confined by the remaining cell wall fragments until these disappeared. Walls of protoxylem cells might remain intact for a time after the syncytium had surrounded them, but eventually they also disintegrated. They were readily dislodged and crushed when the roots were sectioned.

Before the larva underwent the second molt, the walls of most host cells close to the head were completely dissolved and their protoplasm combined into the developing syncytium which might already be 600 to 700 microns long. The cytoplasm was comparatively thin at this stage,



Part of a developing syncytium in a longitudinal section of a Ladino clover root which showed extensive dissolution of cell walls and also showed some deeply stained persistent wall fragments. At center and right, the cytoplasm showed a striated appearance, and nuclei were elongated in the direction of the striations. At far left (arrow), parts of three tubular filaments were faintly discernible. ×500. (Fig. 8)

and the enlarged nuclei accumulated near the head of the larva. Extension of the developing syncytium usually was most rapid away from the root tip.

Syncytia associated with third- and fourth-stage larvae. The syncytium elongated progressively as the nematode grew, attaining lengths up to 1.4 mm. by the eighth day and 1.8 mm. by the twelfth. Later expansion was mainly in diameter, with elongation becoming much slower. When a nematode developed near the apical region of a young root, the entire vascular cylinder near the head of the larva was usually taken up by the syncytium as early as the eighth or tenth day of development.

The cells near the syncytium continued to differentiate and mature as it developed, and metaxylem elements were among the last cells to be included in the syncytial mass. Strands of metaxylem often occurred structurally intact, completely enclosed within a young syncytium (Figs. 6-B, C). Later, however, most of these structures also disintegrated. Portions of the walls were dissolved away; the elements ended

abruptly in syncytial protoplasm; and isolated elements and fragments occurred within the syncytium. In more mature roots with diarch and triarch xylem, the metaxylem often delimited the syncytium, which was composed mainly of what had been phloem cells. This metaxylem might persist as intact vessels, possibly with small portions of the walls dissolved and with the vessels somewhat infiltrated with syncytial protoplasm, or only as a barrier composed of the walls originally opposite the syncytium, the lumen of the vessels being occupied by the syncytial protoplasm. These syncytia had a somewhat irregular outline depending on the extent to which the confining xylem strands had been invaded. Very commonly the metaxylem strands exhibited abnormal staining reactions some distance ahead of the elongating ends of the syncytium.

The syncytia had no clearly defined thickened lateral walls in early stages, yet the development was definitely limited by the endodermis (Figs. 4, 5) or, in some cases, the cortex. As a nematode approached maturity, however, the lateral walls of the syncytium were generally smooth and might become thickened, but they were not always continuous, being sometimes broken by areas through which the syncytium still was encroaching upon neighboring cells. The elongating ends of syncytia showed no clear demarcation until the nematode was fully mature and even then they were never completely delimited. The thickening of the wall around the stylet usually occurred at an early stage. This wall stained vividly with safranin and stained differentially with other cell wall stains. In some specimens, however, it remained relatively thin during the entire period of development.

Some dividing cells, probably of pericyclic origin, often occurred at the edge of a syncytium. These were found either as a very small group in young roots or in a band almost completely encircling the syncytium in more mature roots. The greatest amount of new tissue produced in this way usually occurred between the syncytium and the body of the nematode (Fig. 5), where it contributed, along with the increase in the size of the nematode itself, to forcing the parasite to the surface of the root. Parenchyma cells of the pericycle surrounding the syncytium occasionally differentiated into tracheal elements with scalariform thickening and with a rather disorganized continuity (Fig. 7).

The protoplasmic contents of syncytia became progressively denser, were more coarsely granular, and stained deeper in the later stages, with very irregular clear areas commonly occurring. The cytoplasm often presented a streaked or striated appearance (Fig. 8) which sometimes followed the contours of wall fragments that projected into the syncytial mass, as though fluid currents occurred in the syncytium. This was

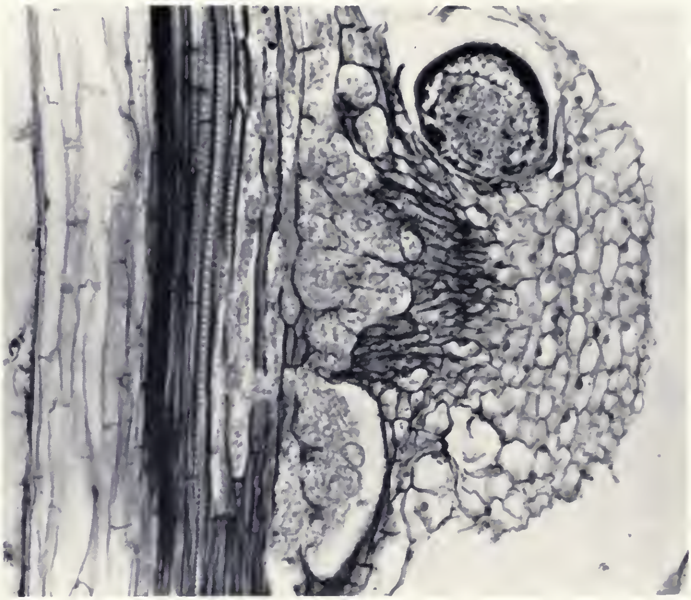
observed even when several chemically unrelated fixatives were used. Nuclei also tended to be oriented as if by currents. The contents of the cytoplasm were observed to be of a rather heterogeneous nature when viewed under high magnification. No definite cell membrane could be seen encompassing the protoplasm, but plasmolyzed areas of well developed syncytia had smooth and regular contours. The characteristic protoplasm of syncytia faded almost imperceptibly into normal tissue at advancing peripheral areas where it also often extended into the lumen of xylem vessels.

Syncytia associated with mature females. During the period when the female was releasing eggs, the syncytium grew very slowly until finally expansion stopped and deteriorative changes took place. The lateral walls usually thickened somewhat during this stage, but the areas farthest from the head still remained relatively undefined with no distinct walls separating them from adjoining cells. The central portion of the syncytium became more or less free of cell walls. Only a few thin membranous fragments separated some parts.

Apparently when the nematode ceased to feed, the syncytial cytoplasm became alveolar and contained many small globules. Remaining nuclei became pycnotic, with deeply staining membranes, coagulated strands of nucleoplasm, and distorted nucleoli. These changes in Ladino clover were much as shown for pea in Figs. 16 and 17. Some nuclei of normal size and otherwise similar to those in unaffected cells, were found within these syncytia. Such nuclei either had not undergone hypertrophy or had returned to their original size and shape because of the diminution or cessation of the stimulatory effects of the nematode's feeding. The cytoplasm eventually disappeared, and its coarsely granular inclusions and cell wall fragments accumulated against the walls. The contents of dead syncytia stained heavily with safranin, as is typical of necrotic cells. Xylem elements at the ends of the syncytia stained similarly, indicating that the effects of feeding had spread beyond the confines of the syncytium itself. If the roots were not killed, new growth eventually crushed and replaced the pathological area and sometimes helped to force the cyst to the root surface.

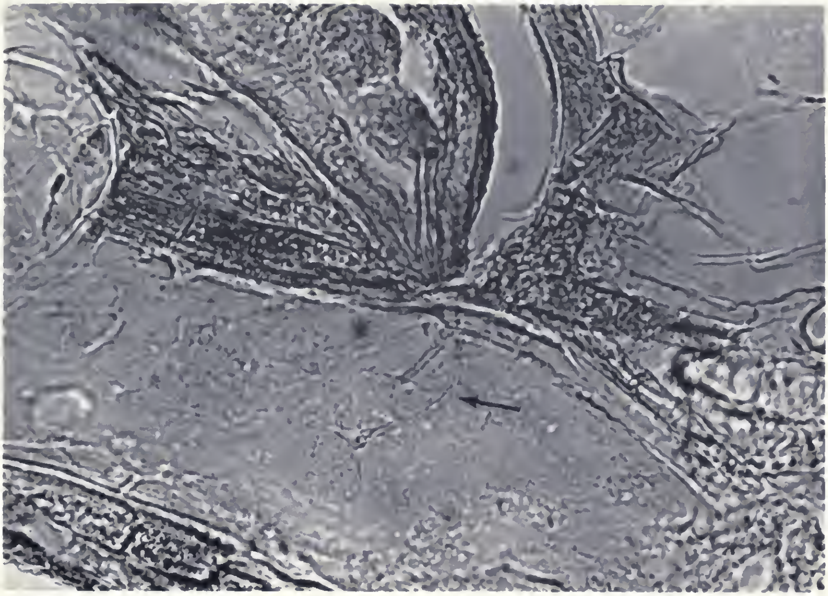
Development in roots with secondary growth. Larvae which entered roots after secondary growth had begun usually migrated into the pericycle where they initiated essentially the same sequence of events in forming syncytia as in younger roots. In secondary tissues the syncytia developed almost entirely from phloem cells. At least a few xylem elements were invaded, however, causing some vessels to open into the syncytial mass. Division of pericycle cells adjacent to a syncytium

caused it to be more definitely delimited. The increased secondary thickening of cell walls in more mature roots resulted in a less complete dissolution of the walls of the cells involved, giving the syncytium a very irregular, broken appearance (Fig. 9). The total volume of the syncytium was similar to that formed in primary tissue.



Longitudinal section of a relatively mature Ladino clover root at the point of emergence of a stunted lateral root, containing a section of an immature female (upper right). Contrast the irregular form of this syncytium with the one in the younger root shown in Fig. 5. This nematode probably entered through the cortical wound made by the emerging lateral. An adjacent section showed its head was near the lower lobe of this syncytium. ×240. (Fig. 9)

Tubular filaments in syncytia. Small, hyaline, tubular structures occurred within the syncytium, close to the tip of the stylet, that evidently were related in some way to the nematode's feeding (Figs. 7, 8, 10). Sometimes one was attached to the tip of the stylet. The tubes were usually 50 to 70 microns long and about 2 microns wide throughout their length, and had circular cross sections. They were rarely if ever straight, but curved gently and sometimes were distinctly coiled. Generally a syncytium contained several tubes distributed so as to suggest that they had formed successively at the stylet, became dislodged, and had been carried away by movement of the cytoplasm.



Longitudinal section of a Ladino clover root, processed 37 days after entry of the nematode, showing the anterior end of a mature female, above center, and part of a syncytium with sparse cytoplasm below. Two tubular filaments (arrow) lay in the syncytium near the head of the nematode, both of them having one end in contact with the cell wall through which the nematode fed. The distal end of the longer tube was in contact with a faintly visible nucleus. ×720. (Fig. 10)

These tubes were observed as early as 6 days after a nematode had entered the root and at almost all stages after that. They also occurred in pea, red clover, and spinach.

In cytological preparations the tubes were detected mainly by their refractive properties. It was not possible to stain them differentially, although they often were clearly visible under high magnification. Their nature was not determined, but it seems probable that they consisted either of secretions injected by the nematode through the stylet or of products of reactions between such secretions and the surrounding host cytoplasm.

Nuclear behavior. As the developing syncytium was viewed from its advancing margin toward the region of the nematode's stylet, the nuclei appeared to exhibit a progressive transformation that, with age, terminated in disintegration. In the cells at the edge of the syncytial mass, the nuclei were just being affected and enlarged rapidly. The

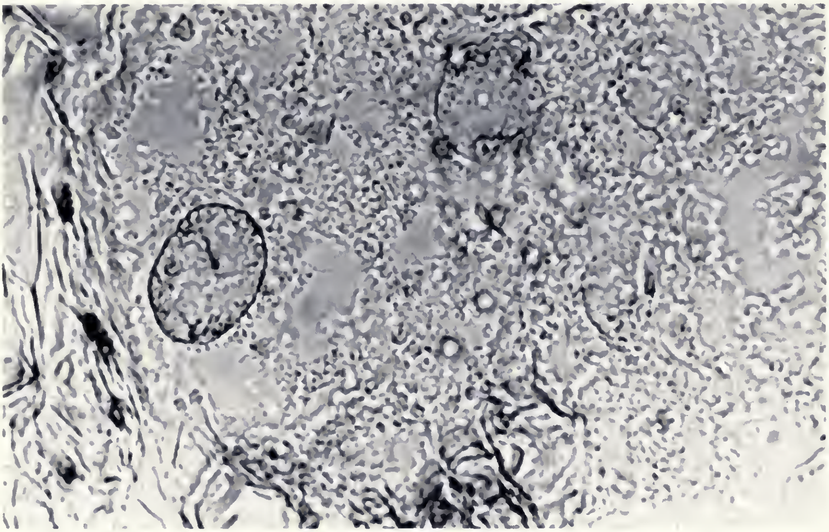
nucleolus and the clear area surrounding it, as well as refractive bodies within the nucleolus, enlarged proportionally. These changes began before there was any obvious breakdown of cell walls. Later, hypertrophy of the nuclei became greatest in close proximity to the stylet. The nuclei apparently were brought there by movements of the protoplasm. These nuclei first enlarged while retaining their normal spherical or pointed-elongate form, depending upon the type of cell from which they originated, and then tended to become bluntly elongate. They might later become very irregular in outline. The nuclear membrane became faint and finally discontinuous as disintegration became complete. Most disintegrating nuclei were found close to the point of stylet insertion but, at times, some were a distance from this point.

A similar progressive response was exhibited in respect to staining. The nuclei close to the stylet region generally stained less readily with nuclear dyes. Although the nucleoli of the nuclei near the margin stained vividly by safranin, their coloration was less intense than that of nucleoli in unaffected cells. The nucleoli became progressively fainter in the vicinity of the stylet. Nuclear membranes also became progressively fainter until a definite nuclear outline was no longer visible.

Syncytial nuclei gave an interesting response to the Feulgen reaction. Many of the greatly hypertrophied nuclei stained positively but fainter than normal nuclei, others stained very faintly, and still others did not react at all. Since the Feulgen stain is generally regarded as an indicator of desoxyribose nucleic acid, there apparently was a gradual destruction of the DNA content of these nuclei. Fig. 11 shows the gradient in staining; deeply staining small nuclei occurred in normal cells close to the edge of the syncytium, and the large hypertrophied nucleus close to the edge was well stained while three others were faintly stained or unstained.

No nuclei within a syncytium were observed to fuse or to undergo division. In somatic cells in the vicinity of syncytia, however, mitotic figures were seen, indicating that the fixation and staining were satisfactory for the detection of such occurrences. Disintegration of some nuclei occurred continuously in a syncytium, with the result that the number of nuclei within syncytia associated with adult females was less than the number of cells included in the syncytia.

Some remote effects of syncytia. The developing syncytium occupied what would otherwise have been vascular tissue. There it evidently restricted translocation and interfered with normal development of the distal parts of a root. Such disturbances appeared to be proportionately more severe the earlier the developmental stage of the



Part of a syncytium stained with the Feulgen reaction. At left, the small, normal nuclei in the cells surrounding the syncytium were deeply stained. Within the syncytium, 4 greatly hypertrophied nuclei were stained variably: the one at the left was well stained, one was faintly stained, and two others were essentially unstained. ×800. (Fig. 11)

rootlet at the point of establishment of the parasite. When nematodes entered relatively mature roots and incited syncytia that occupied only part of the cross section of the stele, the effects on distal growth were mild. When, however, the site of establishment was in a young rootlet where only primary tissues had differentiated and, especially, where the syncytium occupied the entire cross section of the stele, the consequences were much more severe. Cambial activity in the distal parts usually was slowed or stopped. Even the apical meristem sometimes appeared to be stopped, differentiating as parenchyma. Slender rootlets might be visibly narrower below the syncytium (Fig. 20), but it was not clear that necrosis of tips resulted unless complicating factors occurred. Conspicuous development of branch roots was not found in association with syncytia.

No demonstrable hypertrophy of cells surrounding the syncytium was found, and there was no gall formation. Cells in the cortex sometimes failed to elongate normally, and their smaller size gave the impression of increased numbers. In the older roots there sometimes was increased periclinal cell division near a syncytium after its lateral expansion had stopped.

Effects of cool temperatures. Development of the nematode was delayed considerably in Ladino clover seeded and maintained under fluorescent lighting at 15° to 17° C. In three pots of steamed potting mixture infested at the time of seeding with 75 large cysts each, the roots were only lightly infested after 6 months, and all of the normal sized females filled with eggs were still white. One pot contained mostly immature nematodes and a few gravid females. In the other two pots, the nematodes exhibited a wide range of developmental stages.

Sections of infested roots showed that syncytia associated with nematodes in this environment differed in no significant morphological detail from those which developed at greenhouse temperatures. However, it was noted that many of the larger syncytia were shared by several females, all of which appeared to be developing normally. The sharing of a syncytium was an exceptional occurrence at higher temperatures. Host tissue in the vicinity of most of the syncytia had matured, and root elongation and cambial activity were generally not appreciably restricted. The pathology was much less severe than in the usual greenhouse environment.

Unfortunately it is not known how much these results were determined by low temperature and how much by weak illumination. The temperature, equivalent to 59° to 62.6° F., is very favorable to *Heterodera rostochiensis.* Chitwood and Buhrer (2) reported mass invasion of potato roots by that nematode at 60° to 61° F. and presented data indicating good development after entry. Ferris (9) found 65° F., the lowest temperature he studied, to be essentially equal to 75° F. in numbers of larvae entering potato roots, in developmental rate, and in early production of eggs. Unless *H. trifolii* is adapted to higher temperatures than *H. rostochiensis,* the dim light may have had more effect than the low temperature on the slow development of nematodes and on the sharing of syncytia by more than one female.

DEVELOPMENT IN SOME OTHER PLANTS

Pea

On the basis of the population of *H. trifolii* studied, the pea may be said to be only a moderately suitable host for this nematode. Larvae entered the pea roots very freely and soon began to feed, but relatively few of them developed to maturity and produced eggs. Some of the mature females that developed in pea roots were comparable in size to those that developed in Ladino clover, but many were stunted and contained few eggs, with the result that multiplication in pea was not

great. Variable development of the nematodes seemed to be closely related to variability in the development of syncytia, and because the necrosis of the syncytia of an immature nematode caused premature death of the nematode, many of the nematodes that invaded pea roots died prematurely.

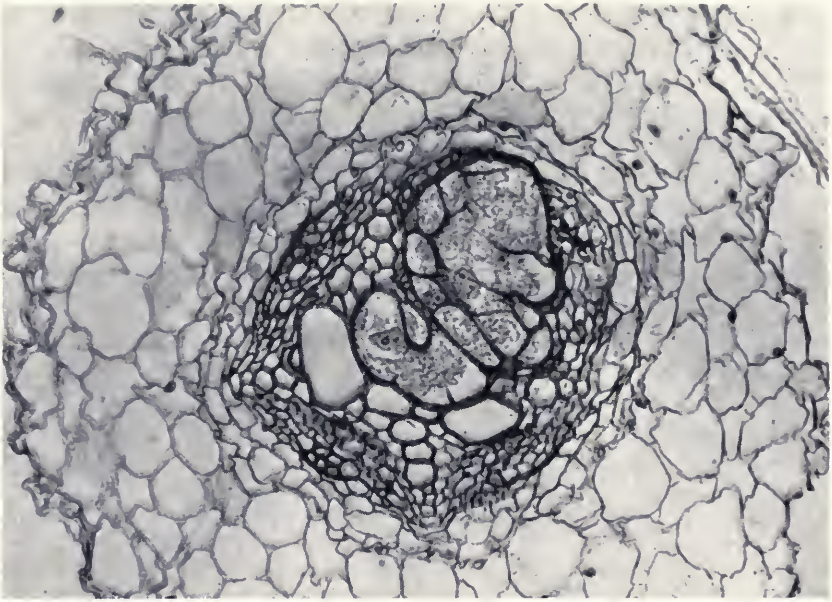
Larvae entered pea roots at almost any point for many centimeters behind the apical meristem and soon stopped wandering and began feeding. This was in sharp contrast with prolonged periods of wandering in red clover. Those that penetrated through the thick cortex of the upper primary root sometimes entered radially in a nearly straight line and established permanent feeding sites as soon as the head reached the endodermis.

Normal syncytia associated with the mature parasite in pea differed in some details regarding size and position from those in Ladino clover. They somewhat resembled syncytia caused by *H. rostochiensis* in potato and tomato (7, 8). They did not develop as rapidly as in Ladino clover and their ultimate size was smaller, seldom exceeding a length of 1.4 mm. Even the largest usually occupied less than a third of the cross section of the stele (Figs. 12, 13). Their ends were more definitely delimited than those in Ladino clover (Fig. 16) though some undelimited regions were always present as long as the nematode continued to feed. The hypertrophied nuclei within the syncytia often contained 2 nucleoli and were as much as 4 to 5 times as long as wide. Incomplete dissolution of walls formed striking patterns (Fig. 14).

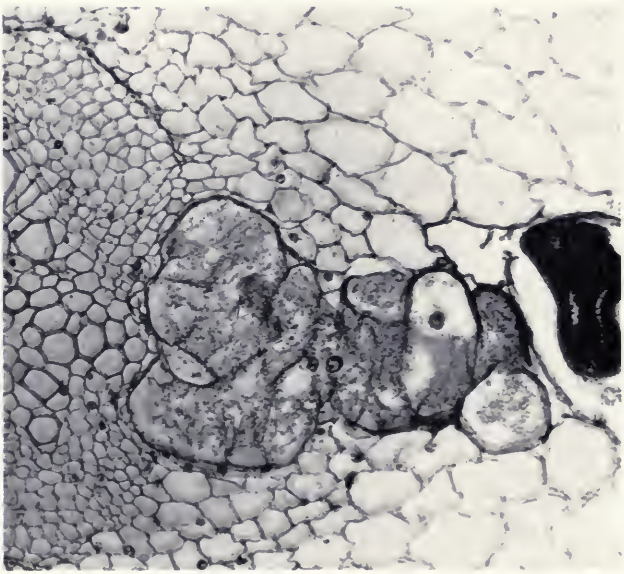
In roots fixed for sectioning at 2-day intervals from infestation until the nematodes were mature, some nematodes at each stage developed normally, inciting normal syncytia, while others were associated with necrotic syncytia and were either dead or about to die very soon. After only 2 days, dead larvae were found surrounded by necrotic cells adjacent to the stele, a condition not found with Ladino clover.

During the first few days after infestation, many dead root tips were found in which larvae could not be recognized, but larvae were found in partially necrotic tips and in tips that appeared normal. The amount of mechanical injury attributable to the wandering of larvae generally appeared small compared with the size of root tips. Death of these tips thus appeared to have resulted from hypersensitivity.

Necrosis of partly formed syncytia and of surrounding cells at later stages also appeared to have resulted from extreme sensitivity of the protoplasts to injections from the nematodes (Fig. 15). Excessive crowding of the parasites was not the controlling factor; dead larvae



Syncytium entirely enclosed within the stele of a pea root. This root was of smaller diameter than the one shown in Fig. 13. Much of the vascular tissue was unaffected. ×240. (Fig. 12)



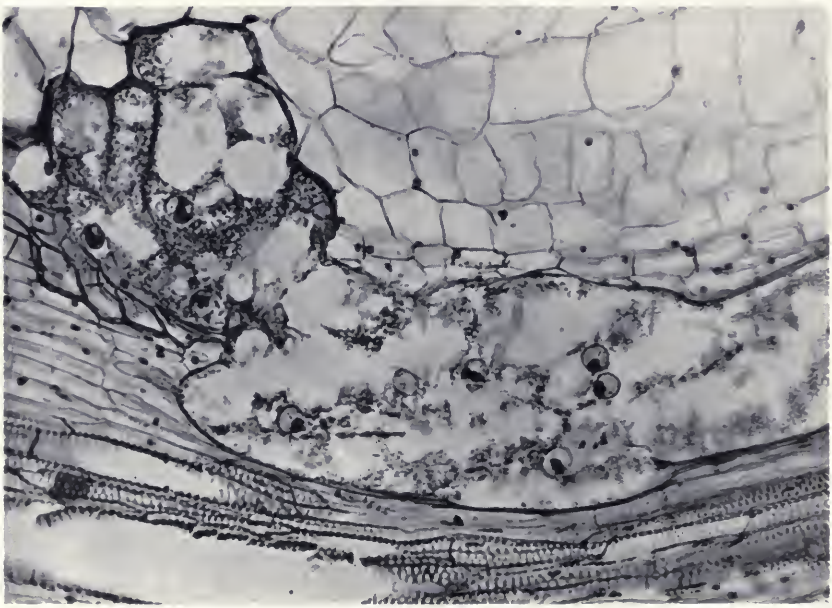
Transverse section of a pea root showing a syncytium that was initiated far out in the cortex but that extended radially inward through the pericycle to include some vascular tissues. ×240. (Fig. 13)

Partial dissolution of cell walls in a syncytium in a pea root processed 16 days after the entry of the nematode. The head of the nematode lay to the left of the area shown in this slightly diagonal longitudinal section. Adjacent sections showed the extension of the syncytium to the head. ×200. (Fig. 14)

Starved nematode in a longitudinal section of a pea root, 20 days after entry. Vascular elements at both ends of the small syncytium were necrotic, and the syncytium was empty. ×240. (Fig. 15)

with necrotic syncytia sometimes were widely spaced, sometimes one thriving nematode with its normal syncytium was found in close proximity to several dead ones, and sometimes several nematodes were found developing successfully in a group with no necrotic cells in the vicinity. Just what determines the variable reaction of pea root cells to individual nematodes is not clear.

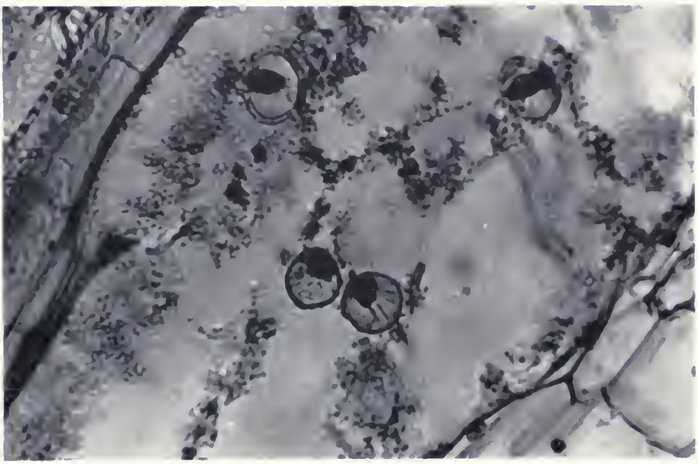
More frequently in pea than in some other plants, the nematode initiated development of a syncytium in the cortex, and this commonly resulted in poor development and the early death of the nematode. Cortical syncytia sometimes included a large number of cells and the walls of these cells disappeared much as in stelar syncytia, but the protoplast remained very sparse and probably had only low capacity for supplying nutrients to the nematode. Development might progress normally where a syncytium arose in the cortex if it rapidly expanded to include cells of the central cylinder and if it made contact with vascular elements (Figs. 13, 16). Otherwise a cortical syncytium might be exhausted and the immature nematode die while adjacent cortical cells remained non-necrotic and appeared normal.



Longitudinal section of a pea root showing part of a degenerating syncytium that was associated with a mature cyst. The syncytium was initiated in the cortex. Longitudinal extension occurred only after it expanded into the stele. ×240. (Fig. 16)

Most of the syncytia in pea, as in other plants studied, arose just inside the endodermis and extended inward into the stele. But even in the stele of the pea, a necrotic reaction frequently was observed involving the syncytial protoplast and some surrounding tissues. Surrounding xylem vessels became swollen and plugged, and cells at the ends of syncytia turned deep brownish-red when stained with safranin. The nuclei of these cells appeared to be dead. Necrosis commonly was followed by activation of a wound cambium that surrounded the necrotic tissue, crushed it, and filled in with new cells.

After a nematode had matured and stopped feeding, the syncytium in pea showed degenerative changes like those described for Ladino clover, the cytoplasm becoming sparse and alveolar and the nuclei pycnotic (Figs. 16, 17).



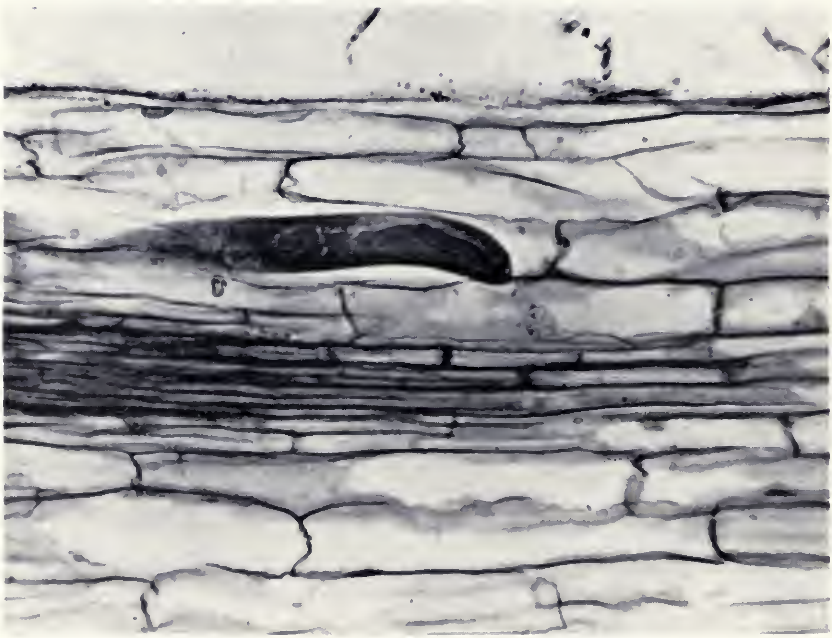
Part of the degenerating syncytium shown in Fig. 16, associated with a mature cyst. Feeding apparently had stopped, and cytoplasmic contents were very sparse. Some cell wall fragments and a few pycnotic nuclei remained. ×560. (Fig. 17)

Red clover

Red clover proved less suitable as a host than pea and much less suitable than the white clovers. Although there were marked differences between individual plants of the composite variety, Midland, in most plants only an occasional nematode developed to as large a size as the average in Ladino clover. The majority of those that developed to maturity were stunted and contained few eggs, and far greater numbers of nematodes died in red clover before reaching maturity compared with

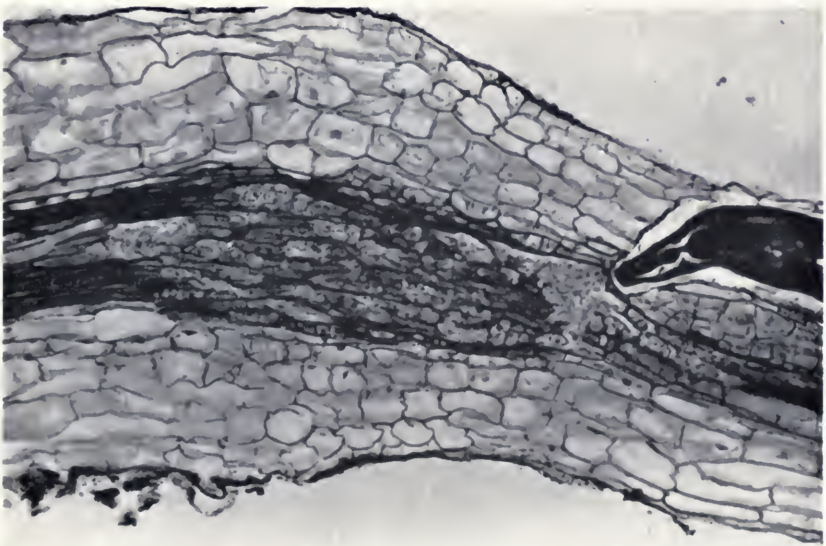
the numbers that died in Ladino clover. Failure to stimulate development of adequate syncytia seems chiefly involved here, in contrast with with early necrosis of syncytia in the pea.

Larvae entered and moved about within roots of red clover much as described for Ladino clover, causing similar injury to young tissues. Passage of several larvae through a root tip might kill it and, as with Ladino clover, vigorous branch roots commonly arose behind such tips. An important point of difference, however, from Ladino clover and other plants studied, is that larvae were very slow to establish permanent feeding sites. Many larvae that appeared to be well supplied with nutrient reserves were still wandering through cortical tissue as long as two weeks after their initial entry. This suggests that these larvae obtained substantial amounts of nutrient by transient feeding from cortical cells, and it indicates that they long failed to obtain whatever stimuli are required to terminate their wandering and establish at permanent sites. Apparently the red clover cells did not readily respond to the feeding of these larvae with the development of syncytia, although some syncytia began to form in the cortex (Figs. 18, 19).



Heterodera trifolii larva in a red clover root approximately 24 hours after entry. It had begun to feed from a cell of an inner layer of the cortex. The nucleus of this cell was markedly hypertrophied. ×540. (Fig. 18)

Heterodera trifolii larva which grew only slightly during 16 days in a red clover root. The syncytium, which was initiated in the cortex, was very small for this age, showed limited cell wall dissolution, a few hypertrophied nuclei, and relatively sparse cytoplasm. ×540. (Fig. 19)



Large syncytium of labyrinthine appearance within the stele of a red clover root processed 12 days after entry of the nematode. Cell wall dissolution was complete near the head of the parasite, but elsewhere it was only sufficient to form passageways connecting all parts of the syncytium. Note the narrowing of the root to the right of center. ×220. (Fig. 20)

Weak development of syncytia which, in turn, was related to slow development and to stunting or to early death of the nematodes in red clover, appears to have been closely related to, if not caused by, slow and incomplete dissolution of cell walls. The largest syncytia in this host were as large as those in Ladino clover and were associated with nematodes that had developed at a normal rate and to large size. Such syncytia, however, contained fewer nuclei and showed less complete dissolution of cell walls than the syncytia in the more suitable host (Fig. 20). At the other extreme, some syncytia remained small with sparse protoplasm, incorporating very few cells and aborting before the nematode matured. Other syncytia that appeared to have developed normally for a time, later became walled off by activities of a surrounding wound cambium. The cambium prevented access to a continuing nutrient supply and resulted in exhaustion of the syncytium and starvation of the nematode. Hence the nematodes died early either from an extreme reactivity of the host tissue or from too slow a dissolution of host cell walls and expansion of the syncytium.

The numerous stunted nematodes that attained maturity in red clover were associated with small syncytia and with very irregular contours. These syncytia contained proportionately few nuclei and much undissolved cell wall material, but their cytoplasm and nuclei appeared essentially the same as in Ladino clover.

Soybean

The development of *Heterodera trifolii* in 27 varieties of soybean was studied in tests in which seed was planted in heavily infested soil. Some of the findings have been published (22). All of these varieties proved much less suitable as hosts than even pea or red clover, yet this study afforded interesting information on the biology of the parasite in plants unsuitable as hosts.

Soybean roots that were stained and cleared after 18 days' growth in infested soil indicated that the larvae entered all varieties freely. Roots treated similarly at the end of the test, after 36 days' growth, still showed some larvae in root tips. They also contained many larval tunnels made by larvae that had either left the roots or died within them. Dead root tips were often encountered which probably had been killed by large numbers of larvae.

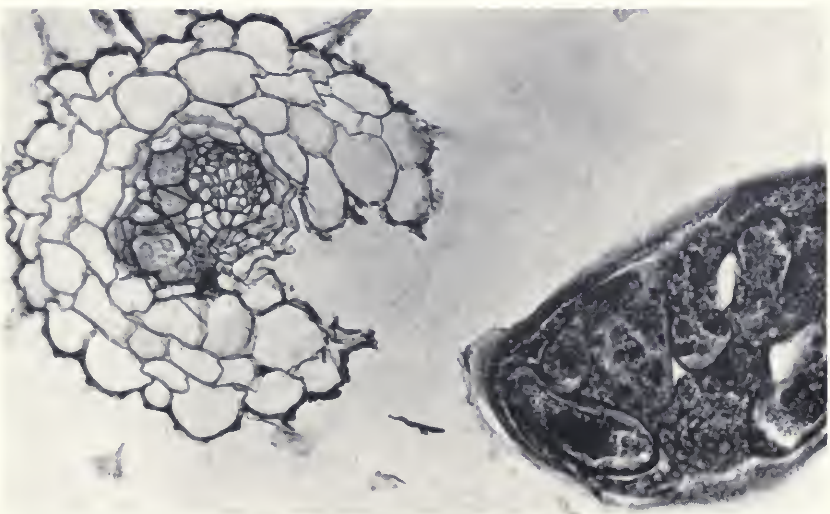
Washings from the soil from each pot at the termination of each test contained several hundred larvae that were caught on a No. 325 sieve. A few of these larvae were well supplied with opaque reserves suggesting that they had hatched recently. A great majority, however, had the starved appearance of larvae that had hatched some weeks

earlier and that had been unable to feed effectively. The presence of the larvae in the soil indicated that the soybean roots were constantly exposed to infestation.

At the close of the test, no enlarged females or cysts could be found in 19 of the varieties. Roots of 6 varieties carried only a very few slender, stunted, immature females which contained no eggs. Mature females or young cysts were found in small numbers only on the roots of the other two varieties, Earlyana and Dunfield, and these were associated with larger numbers of females that had failed to mature.

Most of the advanced stages found had developed slowly, were small and white, and contained relatively few eggs (Fig. 21). No evidence was found of egg deposition in a gelatinous matrix. Development appeared somewhat better in Earlyana than Dunfield, as judged by slightly more numerous and larger cysts, the largest of which were comparable in size to those found in Ladino clover. Maximum development of syncytia in soybean is illustrated in Fig. 22.

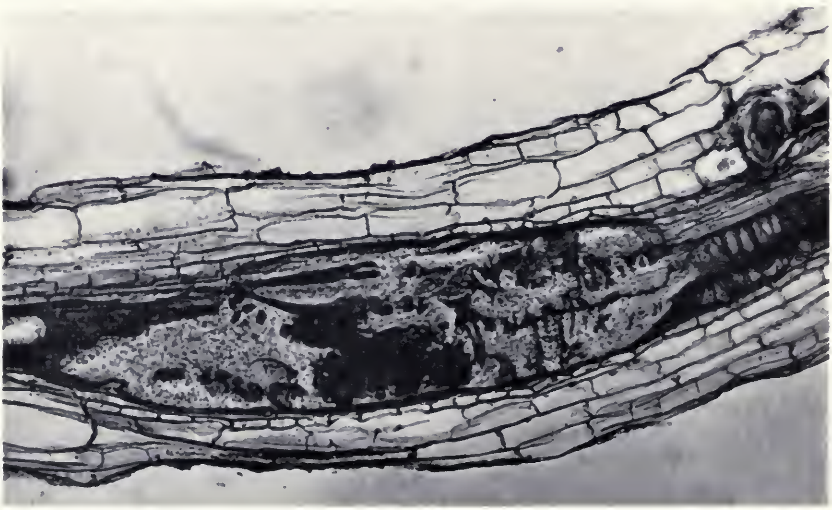
The majority of females which had reached maturity were small and were associated with extremely small syncytia though they had been able to produce a few eggs. Many of these syncytia consisted only of incompletely dissolved portions of a few xylem and phloem elements and of the parenchyma cells between those elements and the nematode's head. The syncytia associated with all of the enlarged



Small syncytium with limited cell wall dissolution in a rootlet of a Dunfield variety soybean, associated with a stunted, gravid *Heterodera trifolii* female that was displaced in processing. ×220. (Fig. 21)

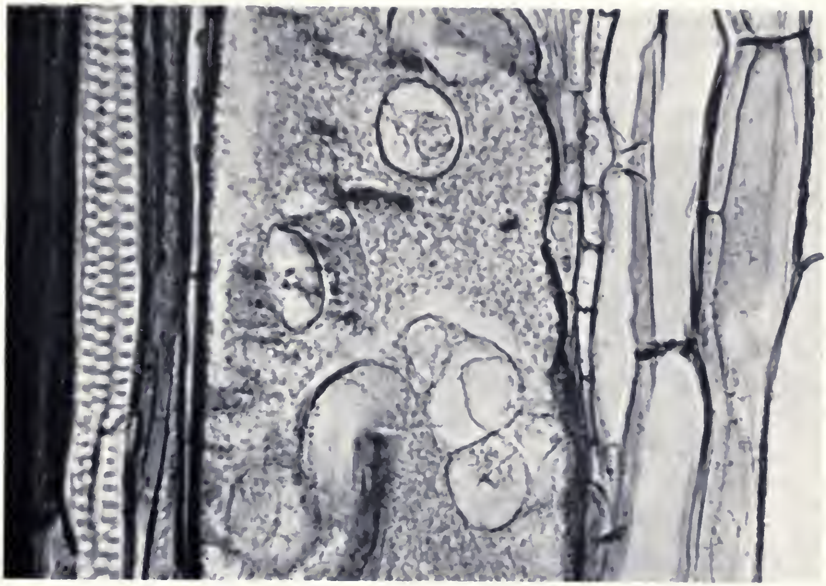
nematodes had some contact with vascular tissue. These syncytia contained the characteristic granular and hyperchromatic cytoplasm but very few nuclei. They also contained greatly thickened and swollen walls that stained differently from normal walls (Fig. 22).

In the few instances in which females developed to a size comparable to those parasitizing Ladino clover, the syncytia were as large as in suitable hosts. Only very rarely, however, did syncytia extend across all of the stele and this was seen only in very slender roots (Fig. 22). While the largest syncytia in soybean were similar in general form to those occurring in Ladino clover, these also differed significantly in the fate of cell walls and in the condition of the nuclei. Swollen wall fragments within the syncytium evidently broke off, floated free in the cytoplasm, and aggregated in masses. The size of these masses was such that apparently very little of the wall material had actually dissolved. Relatively few nuclei were found, and most of these had coagulated nucleoplasm and degenerated nucleoli; almost all were greatly hypertrophied. Some of the largest appeared to be composed of several empty compartments (Fig. 23). Those nuclear membranes which were intact generally stained heavier than the nuclear membranes in Ladino clover syncytia.



One of the largest syncytia found in soybean, containing a large central mass of undissolved cell wall fragments but very few nuclei. Part of the nematode may be seen at the upper right, toward the root tip. In other sections, the syncytium was seen to extend to the head of the nematode. Note the shortness of the cortical cells toward the right end. ×220.

(Fig. 22)



Greatly hypertrophied, degenerating nuclei and scattered cell wall fragments in a syncytium in an Earlyana variety soybean. ×540. (Fig. 23)

Other hosts and resistant plants

Spinach, *Spinacia oleracea,* is a satisfactory host for *H. trifolii* and one that it has in common with the sugar beet nematode, *H. schachtii.* The pathological effects of these two parasites were found to be cytologically indistinguishable in spinach, both of them inciting a development of syncytia very similar in rate and in appearance to the development of syncytia in Ladino clover.

H. trifolii was observed to develop moderately well in individual plants of *Lespedeza cuneata* G. Don. and in yellow sweet clover, *Melilotus officinalis* (L.) Lam., and very poorly in other plants of both species. Development of the nematodes occurred readily in the mature tissue of some plants, but there was no indication that the presence of syncytia prevented the maturation of root tissue in lespedeza and yellow sweet clover. Cytologically, the syncytia formed in both of these species were similar, except in ultimate size, to those in mature Ladino clover roots. The females found in the roots were in various stages of development, some being about as large as those occurring in Ladino clover and filled with eggs, but very few turning brown despite a long period of culture adequate for the production of several generations of the parasite in a favorable host.

No development of *H. trifolii* was obtained in roots of alfalfa, cabbage, carrot, sugar beet, tomato, Kentucky bluegrass, corn, oats, spelt, wheat, or onion. Larvae freely entered the roots of the dicotyledonous plants tested but not those of the monocotyledons.

New host record. Rooted cuttings of smartweed, *Polygonum persicaria* L., potted in steamed soil, were each infested with 500 *H. trifolii* larvae. After 33 days, roots were found to bear large numbers of white females filled with eggs and comparable in size to females of the same age in Ladino clover. This host has not been previously reported for *H. trifolii* and is one that it has in common with the little known species, *Heterodera weissi* Steiner, 1949, which apparently attacks only plants of this genus.

DEVELOPMENT IN ASSOCIATION WITH *MELOIDOGYNE HAPLA*

A cytological study was made of Ladino clover roots grown in soil containing both *Heterodera trifolii* and the northern root-knot nematode, *Meloidogyne hapla.* These parasites were sometimes found together within individual root galls.

M. hapla caused the formation of small galls from which branch roots arose. The nematode was within the gall with its head in the stele surrounded by about four giant cells. The lateral roots arose in the pericycle near the giant cells, and their steles connected with the main vascular cylinder through a network of vascular elements surrounding the giant cells. The galls began to form with the hypertrophy of cortical cells in the vicinity of the giant cells, and continued to increase in size, as the nematode enlarged, by means of the division of parenchyma cells in the pericycle and in the cortex around the body of the nematode.

The giant cells of *M. hapla* contrasted strikingly with the syncytia of *H. trifolii* (Fig. 24). The giant cells did not have well defined walls in the early stages of development, but at later stages they generally had smoothly rounded contours with continuously thickened walls and with disintegrating nuclei clustered irregularly in their centers. Their cytoplasm was highly alveolar and more finely granular than that in the syncytia of *H. trifolii.*

The *H. trifolii* females occurring in the galls, however, were associated with the usual pathological disturbances: the syncytia did not differ from those which occurred when the root-knot nematode was absent. Syncytia were found partially surrounding giant cells, having

invaded xylem, phloem, and parenchyma cells until a single wall separated the two types of pathological tissue (Fig. 24). Sometimes parts of this separating wall appeared to have been dissolved away without the protoplasmic contents of either syncytium or giant cell having been altered and without any evidence of an intermixing of protoplasts.

Giant cells and a syncytium lying in contact within the stele of a Ladino clover root invaded by both *Meloidogyne hapla* and *Heterodera trifolii*. At center and right are 4 giant cells with typically alveolar cytoplasm. In 2 of them the nuclear material is clumped in the interior. A section of the *M. hapla* is in the upper right of this group. At the left is a crescent-shaped syncytium with partly dissolved cell walls and scattered, hypertrophied nuclei. Part of another syncytium is at the extreme lower left. ×240. (Fig. 24)

No evidence was found, in the material studied, that either species of parasite had influenced the other with respect to selection of feeding sites or with respect to development.

DISCUSSION

Investigations reported here provide a more comprehensive understanding of parasitic relationships of *Heterodera trifolii* to plants than is available for other members of this genus.

Injuries to young roots during entry and intracellular wandering of larvae caused some root tip necrosis in young seedlings of Ladino clover in heavily infested soil. More commonly, however, root tip destruction became an important phase of plant injury only after several generations of developing nematodes had lowered plant vigor and reduced the rate of root growth and had built up high concentrations of larvae in the soil. This type of injury was not limited to suitable host plants but occurred in roots of any plant that the larvae invaded freely; yet plants that are not favorable hosts are rarely exposed in nature to populations of larvae sufficiently dense to cause much injury. Ross (31) mentioned injury in roots of susceptible and resistant soybeans invaded by larvae of *Heterodera glycines*. Linford (19) described related types of root tip destruction by larvae of Meloidogyne sp., and Chitwood, *et al.* (3) reported considerable injury to young peach trees planted in soil heavily infested with a species of Meloidogyne that could not develop in that variety of peach.

After the parasite had begun to enlarge at its permanent site, the most important factor in plant injury appeared to be the interruption of translocation, especially that through phloem, as the syncytium developed. This appeared to be sufficient to account for most of the reduction in distal growth of the root. Withdrawal of nutrients by the parasite also must have tended to weaken the plant, but this probably was minor except in very heavily invaded young seedlings. Little is known about the possible occurrence and extent of systemic disturbances than may be caused by diffusion through the plant of low concentrations of the substances that cause development of syncytia.

In contrast with Meloidogyne spp. in which several to many larvae sometimes occupied a single gall and caused proportionately increased swelling, *H. trifolii* seemed never to develop in compact clusters in young roots despite the observed tendency to enter in groups through wounds. They either dispersed, or their syncytia killed the root before they matured. In young roots where a syncytium might occupy the

entire cross section of the stele and extend longitudinally as much as 2 mm., the opportunity for several larvae to develop close together without extreme competition was slight, and there usually was prompt necrosis of a heavily infested rootlet. Older roots, in which primary tissues had matured and secondary growth had begun, were able to support parasites much closer together, with the syncytia tending to be kept separated by the mature primary xylem.

Active *H. trifolii* larvae observed in association with growing roots responded to the presence of the roots, and responded additionally to fresh wounds, by initiating activities leading to entry. The fact that larvae entered as freely into roots of some plants in which they are unable to develop as into roots of suitable plants shows that this response did not indicate the sensing of a suitable source of food. This is not surprising, for suitability as a host apparently depends much less upon the presence of certain nutrients in normal root cells than upon the interaction between nematode and plant that leads to development of adequate syncytia.

In its feeding habits, *H. trifolii* differs somewhat from Meloidogyne spp. (16, 19). In these studies no feeding was observed on epidermal cells although it was seen within the cortex before the larva had entered full length. When the larva stopped at its permanent feeding site, its head became relatively immobile. All feeding from that time on was through a single cell wall. At each molt the old stylet tip was cast off and the new one penetrated the wall close by its side. This contrasted sharply with the feeding of Meloidogyne females which fed briefly from one cell, then retracted the stylet, turned the head and fed from another, continuing to feed over and over from the several cells within reach of the stylet. Each of the cells fed from by Meloidogyne females evidently initiated development of a giant cell, in contrast with the single cell fed from by *H. trifolii* that initiated development of the single syncytium.

Saliva flow from the stylet of *H. trifolii* was not seen even under conditions of observation similar to those that permitted photographing the saliva of Meloidogyne sp. (16). While the larvae were feeding in the cortex where both the stylet and the median bulb were visible, there appeared to be no delay after insertion of the stylet, such as reported for Meloidogyne and *Ditylenchus intermedius* (15), during which it has been assumed that saliva was injected and allowed to act before pulsation of the bulb began. If *H. trifolii* injects saliva only into living protoplasm and only at the site of permanent feeding, one could not expect to see it with the methods used.

Indirect evidence strongly indicates that saliva actually is injected. The alterations of cytoplasm and nuclei, and especially the hypertrophy of nuclei that occurs very early while cell walls are intact, indicate activity of some diffusible substances, and the progressive dissolution of cell walls indicates strong enzymatic activity of a type foreign to the normal root. The slender filamentous tubes found in syncytial protoplasm in the vicinity of the nematode stylet also are strongly indicative that the nematode injects substances into the syncytium.

These hyaline tubes were seen in many syncytia and in several host species. Although their exact nature and function are unknown, the finding of some tubes apparently attached to a stylet tip and others lying nearby suggests that they arose as a coagulum around the periphery of slender streams of saliva injected through the stylet into the syncytium. Probably they were ephemeral since no greater numbers were found in older than in younger syncytia. The alternative possibility that only a few were ever formed and that once formed they persisted a long time seems improbable in view of their characteristic location near the point of stylet insertion. Possibly these tubes were what Němec (27) first described as large mitochondria but later (28) interpreted as long, filamentous crystals.

The syncytium represents a remarkable type of pathological alteration of host tissues, incited by the parasite and required by the parasite for adequate nutrition. The total quantity of nutrients required for growth and egg production had to be translocated to the immobile parasite, and this evidently was the function of the syncytium. Even in early stages the adequate syncytium included some phloem and, as it enlarged, it made end to end contact with more and more phloem and even some xylem elements. Nutrients had to pass from the interrupted vascular elements into the continuous syncytial protoplast, and then either to diffuse or be carried by streaming of the protoplasm to the vicinity of the stylet. A syncytium began to form before there was recognizable growth of the parasite, and in unsuitable plants, the protoplasmic contents of the syncytium might collapse or necrosis of the syncytium might occur before the nematode appeared to be dead. It seems clear that ability of the nematode to incite development of an adequate syncytium in a given plant was essential to successful parasitism. In moderately suitable hosts as well as in unsuitable plants, the development of individual nematodes and associated syncytia varied highly. Regardless of host, however, the rate of development and the ultimate size of the nematode were closely related to and probably were determined by the developmental rate and final size of the syncytium.

These studies led to the recognition of some of the factors that make certain plants unsuitable as hosts. Among the Gramineae, none of the plants tested was entered freely if at all by the larvae. Some dicotyledonous plants were entered freely, but neither nematodes nor syncytia ever made appreciable development in them. In roots of red clover, some larvae continued to wander through the cortex for as long as 2 weeks, indicating that they did not readily obtain whatever stimulus was required to induce them to establish sites of permanent feeding. Also in this plant, syncytia developed very slowly. The dissolution of walls was slow and incomplete, with the result that most syncytia remained too small to support normal development of parasites. The sparse protoplasm commonly was exhausted before the nematodes matured. In soybean also, most syncytia were of very inadequate size and developed slowly, usually incorporating few cells, with very weak dissolution of walls. In pea, which proved somewhat unsuitable as a host under the conditions of this study, the nematodes quickly initiated syncytia, even within the inner cortex. Development of syncytia in pea tended to be slow, however. In addition, necrosis of syncytia in pea occurred more commonly than in other plants studied in detail. Such necrosis occurred at different developmental stages and sometimes in syncytia that had been developing rapidly, always promptly resulting in the death of the parasite. This appeared to be a type of hypersensitivity to secretions of the nematode. Evidence of a second type of hypersensitivity in pea was seen in the necrosis of root tips through which larvae had wandered. Ross (31) has reported hypersensitivity in soybeans resistant to *Heterodera glycines*. Another tendency seen in some unsuitable plants was the walling off of a syncytium by activity of a surrounding wound cambium.

The unsuitability of pea as a host in these studies probably does not apply equally to other varieties of this plant. Mulvey (23) comparing 5 pea varieties, found very heavy infestation of 2, intermediate conditions in 2, and only light infestation of one. In addition, Mulvey grew his plants in cooler soil, more nearly optimal for pea than the temperatures that prevailed in the present studies. It is possible that even the canners' Perfection pea might have responded more favorably to the parasite at lower temperatures.

For successful parasitism, a delicate balance must prevail between the injections of the parasite and the response of the plant. If the substances injected were toxic to the plant protoplasm, the cells were killed instead of stimulated to develop a syncytium. Enzymes injected by the parasite, or activated by the parasite if they already were present

within the normal root, had to dissolve cell walls at a rate sufficient to allow the syncytium to enlarge fast enough to supply the increasing nutritional needs of the growing nematode, and such wall dissolution soon had to result in adequate contacts with vascular tissue.

It seems remarkable that the required balance was established in unrelated species in distinct plant families and yet was poorly if at all developed in plants closely related to very suitable hosts. That success or failure to achieve this balance could be determined by minor differences in the parasites was shown by Mulvey's work with red clover (23). A Canadian population of *H. trifolii* failed to propagate in this plant, while a population of the same species from England thrived in it in a greenhouse at Ottawa.

From the present studies it is evident that the syncytia incited by *H. trifolii* in the several hosts studied were more distinct in character from giant cells incited by Meloidogyne spp. than earlier studies of Heterodera species would indicate. In function, the two types of pathological tissue are similar, serving to make sufficient food available to the parasites. Although differences in feeding habits apparently account for a single syncytium as compared with a group of giant cells, the differences in appearance of cytoplasm and in the fate of nuclei when the two develop side by side in Ladino clover, are evidence of some major difference in the inciting stimuli.

Meloidogyne distributes its salivary injections and withdrawal of food among several cells and thus allows time between periods of disturbance for each cell to react. This contrasts sharply with the continuous and concentrated disturbance at one point set up by the feeding of *H. trifolii.* Conditions required for establishment of a suitable balance between parasite and plant would appear to be much more critical in the latter. Perhaps in this difference lies a key to the ability of various Meloidogyne spp. to develop successfully in many more hosts than does any known species of Heterodera.

SUMMARY

A comprehensive study was made of the parasitic relationships of the clover cyst nematode, *Heterodera trifolii* Goffart in favorable hosts, in less favorable hosts, and in some highly resistant plants. The objectives were to determine how this parasite enters the plant, how it obtains its food, how it injures the plant, and what kinds of plant responses are required for successful parasitism. The very favorable host, Ladino clover, was studied in most detail.

Living larvae penetrated the epidermis with some difficulty, and only after the stylet had been thrust repeatedly in a way that evidently weakened the outer wall. Once the head was within an epidermal cell, intracellular penetration into the cortex was rapid, the entire body often being inside within 15 minutes. Larvae were attracted to and entered through fresh wounds. They were seen to begin feeding only after entry into the root was almost complete.

Damage to young roots by larvae during penetration and intracellular wandering sometimes was extensive. If several larvae entered close together, they might kill a root tip. Such injury was not limited to host plants but occurred in roots of plants that were entered freely even though they were highly unfavorable for development of the parasite.

Females dissected alive from roots or observed in water exhibited alternating regular periods of stylet thrusting and pulsation of the median bulb. The head was essentially immobile, and all the stylet thrusting was in one direction. The stylet was held far protruded during pulsation of the bulb, and was retracted when pulsation stopped.

Studies of paraffin sections and of whole mounts show that when a larva stopped wandering and established its site of permanent feeding, in a root without secondary thickening, its body usually lay in the inner cortex, close to the endodermis, with its posterior end toward the root tip. Its head usually lay in a broken endodermal cell where the stylet could be thrust through the inner wall into a cell of the stele. Occasionally the head lay in the inner part of the cortex.

A syncytium quickly began to form, starting with hypertrophy of the nucleus and with an increased granularity of the cytoplasm of the cell into which the stylet was inserted. These changes were followed promptly by nuclear hypertrophy in adjoining cells and by dissolution of cell walls that allowed protoplasts to merge. During the life of the parasite, the syncytium continued to enlarge, with progressive dissolution of walls of cells at its margins and with the merging of protoplasts, until a multinucleate, densely granular mass of protoplasm resulted, incompletely divided by remains of partly dissolved walls. If a syncytium was initiated in the stele, it was contained within it, and its greatest direction of growth was away from the root tip. Some syncytia attained a length of 2 mm., and some occupied the entire stele in the vicinity of the stylet. That it had to intercept translocated nutrients from the vascular system in order to supply the parasite with adequate nutrition was indicated by failure of the nematode to thrive when a syncytium arose in the cortex unless it extended into the stele.

Neither conspicuous hypertrophy of cells nor nuclear division was observed within a syncytium. Except in the early stages, the number of contained nuclei was less than the number of cells that had merged with the syncytium, because disintegration of nuclei was a continuing process.

Slender tubular filaments were seen within syncytial cytoplasm in Ladino clover and in several other hosts. They lay close to the stylet and sometimes one appeared to be attached to the stylet tip, indicating that they were related in some way to the feeding process. Frequently several such tubes could be seen lying fairly close together and nearly parallel, which suggests that they had formed successively at the stylet, had become dislodged, and had been moved away by a slow circulation of the protoplasm.

In a canners' strain of Perfection pea, the development of *H. trifolii* varied highly, many individuals dying early because syncytia became necrotic, some remaining stunted in association with slowly developed syncytia, while others were associated with adequate syncytia and developed as well as in Ladino clover. However, a continuous population did not develop in Perfection pea.

In red clover, some larvae continued to wander as long as two weeks. Most of the syncytia that were initiated developed slowly with inadequate dissolution of cell walls, and some of the small syncytia aborted, causing the parasite to die. A few normal parasites and syncytia did develop in red clover.

In tests of 27 varieties of soybean, a very few mature females of normal size were found in only 2 varieties. These were associated with adequate syncytia. The great majority of nematodes that entered the roots of these and all the other varieties remained very small and failed to complete their development. They were associated with small and chiefly abortive syncytia.

Both *H. trifolii* from clover and *H. schachtii* from sugar beet developed very well in spinach and were associated with syncytia that were similar to those described for Ladino clover.

H. trifolii was able to develop to maturity, although usually stunted, in individual plants of *Lespedeza cuneata* and yellow sweet clover. The larvae entered the roots of several additional dicotyledonous plants but did not enter the roots of the six monocots tested.

Regardless of which kind of susceptible plant was the host, the size of the female at maturity was closely related to the size of the syncytium, and the rate of development of the nematode depended upon the developmental rate of the syncytium.

H. trifolii and *Meloidogyne hapla* developed close together in roots of Ladino clover without either parasite appearing to influence the other. The syncytium of the one contrasted strikingly with the giant cells of the other even when the two types of pathological tissue lay in contact.

LITERATURE CITED

1. BERLINER, E., and BUSCH, K. Über die suchtung des Rübennematoden (*Heterodera schachtii* Schmidt) auf agar. Biol. Zentbl. 34:349-356. 1914.
2. CHITWOOD, B. G., and BUHRER, EDNA M. Further studies on the life history of the golden nematode of potatoes (*Heterodera rostochiensis* Wollenweber), season 1945. Proc. Helminth. Soc. Washington. 13:54-56. 1946.
3. CHITWOOD, B. G., SPECHT, A. W., and HAVIS, LEON. Root-knot nematodes. III. Effects of *Meloidogyne incognita* and *M. javanica* on some peach rootstocks. Plant and Soil 4(1):77-95. 1952.
4. CHRISTIE, J. R. The development of root-knot nematode galls. Phytopathology 26:1-22. 1936.
5. DARLINGTON, C. D., and LACOUR, L. F. The handling of chromosomes. Geo. Allen and Unwin Ltd., London. 180p. 1947.
6. DARROW, MARY A. A simple staining method for histology and cytology. Stain Technol. 15:67-68. 1940.
7. FASSULIOTIS, GEORGE. A study of potato roots infected by the golden nematode, *Heterodera rostochiensis* Wollenweber. Unpublished master's thesis, New York University. 1954.
8. FELDMESSER, JULIUS. The life cycle of the golden nematode, *Heterodera rostochiensis* Wollenweber, 1923, on tomato and a cytological study of its effects on the host. Unpublished doctoral dissertation, New York University. 1953.
9. FERRIS, J. M. Effect of soil temperature on the life cycle of the golden nematode in host and non-host species. Phytopathology 47:221-230. 1957.
10. GERDEMANN, J. W., and LINFORD, M. B. A cyst-forming nematode attacking clovers in Illinois. Phytopathology 43:603-608. 1953.
11. GOFFART, H. Untersuchung am Hafernematoden *Heterodera schachtii* Schum unter besonderer Berücksichtigung der schleswigholsteinischen Verhältnisse I. III. Arb. Biol. Reichsanst, Berlin. 20:1-26. 1932.
12. HAGEMEYER, J. W. A new stage in the life cycle of the golden nematode, *Heterodera rostochiensis* Wollenweber. Proc. Helminth. Soc. Washington. 18:112-114. 1951.
13. JOHANSEN, D. A. Plant microtechnique. McGraw-Hill, New York. 523p. 1940.
14. KOSTOFF, D., and KENDALL, J. Cytology of nematode galls on Nicotiana roots. Zentbl. Bakt. Abt. II. B. 81:86-91. 1930.
15. LINFORD, M. B. The feeding of some hollow-stylet nematodes. Proc. Helminth. Soc. Washington. 4:41-47. 1937.
16. ———— The feeding of the root-knot nematode in root tissue and nutrient solution. Phytopathology 27:824-835. 1937.
17. ———— A miniature root-observation box. Phytopathology 30:348-349. 1940.

18. Linford, M. B. Methods of observing soil flora and fauna associated with roots. Soil Sci. 53:93-103. 1942.

19. ———— The transient feeding of root-knot nematode larvae. Phytopathology 32:580-589. 1942.

20. Linford, M. B., and Oliveira, Juliette M. The feeding of hollow-spear nematodes on other nematodes. Science 85:295-297. 1937.

21. Mankau, [G.] R. Pathological disturbances caused by *Heterodera trifolii* in susceptible and resistant plants. (Abs.) Phytopathology 48:395. 1958.

22. Mankau, G. R., and Linford, M. B. Soybean varieties tested as hosts of the clover cyst nematode. Plant Disease Rptr. 40:39-42. 1956.

23. Mulvey, R. H. Susceptibilities of plants to the clover cyst nematode, *Heterodera trifolii,* and the period required to complete a life cycle. Nematologica 4:132-135. 1959.

24. ———— Investigations on the clover cyst nematode, *Heterodera trifolii* (Nematoda: *Heteroderidae*). Nematologica 4:147-156. 1959.

25. Nagakura, K. Über den Bau und die Lebengeschichte der *Heterodera radiciola* (Greeff) Müller. Jap. Jour. Zool. 3:95-160. 1930.

26. Němec, B. Das problem der Befruchtungsvorgänge und andere zytologische Fragen. Teil 4. Vielkernige Riesenzellen in Heterodera-gallen. pp. 151-173. Gebrüder Borntraeger, Berlin. 1910.

27. ———— Über die Nematodenkrankheiten der Zuckerrübe. Zeit. für Pflkrankh. 21:1-10. 1911.

28. ———— Über die Gallen von *Heterodera schachtii* auf der Zuckerrübe. Stud. Pl. Physiol. Lab. Charles Univ. 4:1-14. 1932.

29. Raski, D. J. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. Phytopathology 40:135-152. 1950.

30. Rawlins, T. E. Phytopathological and botanical research methods. John Wiley and Sons, Inc., New York. 156p. 1933.

31. Ross, J. P. Host-parasite relationship of the soybean cyst nematode in resistant soybean roots. Phytopathology 48:578-579. 1958.

32. Sengbusch, R. Beitrag zur Biologie des Rübennematoden *Heterodera schachtii.* Zeit. für Pflkrankh. 37:86-102. 1927.

33. Treub, M. Quelques mots sur les effets du parasitisme de l'*Heterodera javanica* dans les racines de la canne à sucre. Ann. Jard. Bot. Buitenzorg. 6:93-96. 1887.

3M—12-60—72468